THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXXI JULY–AUGUST, 1955 NUMBER 4

MORPHOLOGIC EVOLUTION OF AMAUROTIC FAMILY IDIOCY

THE PROTRACTED PHASE OF THE DISEASE *

STANLEY M. ARONSON, M.D.; BRUNO W. VOLK, M.D., and NATHAN EPSTEIN, M.D.

From the Departments of Laboratories and Pediatrics, Jewish Chronic Disease Hospital (Drs. Aronson, Volk, and Epstein), and the Department of Pathology, State University of New York, College of Medicine (Dr. Aronson), Brooklyn, N.Y.

Amaurotic family idiocy, as originally described by Sachs,[1] designated a clinical entity restricted to infants of Jewish extraction and characterized by a familial tendency, arrest of mental processes, blindness, and progressive weakness. Subsequent observations have led to expanded concepts of the disease. However, while cases have been described commencing in late infancy,[2] during the juvenile period,[3] and even in early adulthood,[4] such instances are extremely rare and the bulk of published reports are concerned with the infantile variety. Little has been added to the initial characterization of the syndrome in the past 70 years to indicate any extensive clinical variability. Similarly, published morphologic studies of necropsy material have displayed considerable uniformity. Most reports have emphasized the lack of gross stigmas, other than non-specific atrophy, confining descriptive data to the microscopic changes.

An outstanding characteristic of the disease, as noted in previously published surveys, is the relatively brief clinical course. Thus, in an extensive review by Globus,[5] comprising 11 cases of amaurotic family idiocy, the average duration was somewhat less than 7 months, and 14 months was the maximum period of survival. Other reports mention a more prolonged clinical course, but approximately 12 to 18 months is the usually anticipated duration in this uniformly fatal disease.[6,7]

The purpose of this report is to present data for a series of 8 patients with amaurotic family idiocy and 2 patients with Niemann-Pick's disease, most of whom have survived for significantly pro-

* Presented in part at the Fifty-first Annual Meeting of the American Association of Pathologists and Bacteriologists, Philadelphia, April 9, 1954.

Received for publication, November 22, 1954.

longed periods as compared with the life expectancy usually associated with this illness. The 2 patients who were designated as having Niemann-Pick's disease were included in this study despite the additional component of extraneural lipid storage, since the central nervous system counterpart does not differ appreciably from the Tay-Sachs variety of amaurotic family idiocy, an observation which led some observers to refer to such cases as amaurotic familial idiocy with Niemann-Pick's disease.[5] In most instances the children were institutionalized shortly after recognition of the disease and remained in the hospital during the entire course of the illness. Eight patients have succumbed and have been necropsied (Table I). The present report is concerned primarily with the collected anatomical data of the necropsied patients and serves to indicate certain morphologic features which have not been generally ascribed to the disease in the past.

Duration

The characteristic symptoms in the 8 patients, all of whom were of Jewish extraction, commenced within the first year of life. Duration, following the onset of detectable illness, varied considerably. It became apparent in retrospect that the extent of survival played a major rôle in determining the anatomical expression of the disease. The cases, for purposes of convenience, were assigned to three groups: (1) patients surviving less than 15 months after the onset of pertinent symptoms (abortive course); (2) patients living from 15 to 24 months after the beginning of the disease (intermediate course); and (3) patients living for periods over 2 years following initial symptoms (protracted course). This classification afforded an opportunity to equate certain phases of the disease process with the relative chronicity and thus to study certain pathologic aspects of the prolonged course of central nervous system degeneration inherent in amaurotic family idiocy. Representative case reports and necropsy findings from each category are presented.

Abortive Course

Case 1

P. M., a white male child, had an uneventful birth and appeared to develop normally during the first 4 months of life. The primary abnormality noted by the parents was listlessness and abdominal swelling. These signs increased insidiously but progressively and in the subsequent months were joined by cachexia. Blindness became apparent toward the latter portion of the first year. Lack of development, hypotonia of all extremities, and severe inanition supervened and the infant died at the age of 17 months. The total clinical course was 13 months. (It is of interest to note that the patient had a twin brother (case 2) who presented an almost identical clinical history and died 1 month after his sibling.)

TABLE I

Data for Ten Patients with Amaurotic Family Idiocy

TABLE 1

Data for Ten Patients with Amaurotic Family Idiocy

Case no.	Sex	Familial incidence	Presenting symptoms	Age at first symptom	Amaurosis (ultimate)	Duration of illness	Increase in cranial circumference[18]	Pneumoencephalography	Age at death	Brain weight	Additional anatomical findings
				mos.		mos.			mos.	gm.	
1. P. M.	Male	Yes	Listlessness, abdominal swelling	4	Yes	13	None	Not done	17	906	Niemann-Pick's disease involving spleen, liver, lymph nodes, bone marrow
2. K. M.	Male	Yes	Listlessness, abdominal swelling	4	Yes	14	None	Not done	18	940	Niemann-Pick's disease involving spleen, liver, lymph nodes, bone marrow
3. S. G.	Male	No	Apathy, progressive blindness	6	Yes	14	None	Not done	20	1,300	Bronchopneumonia
4. M. D.	Male	No	Listlessness, blindness, hypotonia	7	Yes	23	Slight	Not done	30	1,360	Confluent bronchopneumonia, pleuritis
5. R. R.	Male	No	Listlessness, retarded development	5	Yes	31	Marked	Ventricular dilatation	36	1,690	Bronchopneumonia, pleural effusion
6. P. R.	Female	Yes	Convulsions	7	Yes	28	Moderate	Ventricular dilatation	35	1,520	Cachexia, bronchopneumonia
7. R. B.	Female	No	Retarded development, blindness	4	Yes	33	Marked	Ventricular dilatation	37	1,570	Bronchopneumonia, decubital ulcers of scalp
8. B. L.	Male	No	Weakness, retarded development	5	Yes	42	Marked	Ventricular dilatation	47	1,800	Bronchopneumonia, thymic atrophy, esophageal diverticulum
9. S. T.	Female	No	Hyperacusis, irritability, loss of ability to hold head up	5	Yes	16*	Equivocal	Suggestive lateral ventricular dilatation			
10. R. G.	Female	No	Decreased activity, flaccidity	7	Yes	14*	None	Cortical atrophy and mild dilatation of all ventricles			

* Still alive.

Necropsy Findings. At necropsy the brain weighed 906 gm. The investing leptomeninges were diffusely but mildly thickened and opaque. The cerebral gyri were narrowed and the sulci broadened. Atrophy of the cerebellum and brain stem was evident also. Consecutive sections through the cerebral hemispheres revealed a moderate increase in consistency but no other distinguishing feature. Microscopic sections from all parts of the brain showed ballooned neurons distended with a poorly staining, minimally hematoxylinophilic granular substance which displaced the nucleus toward the cell membrane. The abnormal cytoplasmic inclusions were mildly sudanophilic. Nissl bodies were diminished in number or absent. The surrounding interstitium contained numerous rounded microglia. All levels of the neuraxis showed these characteristic ganglionic alterations as well as the responsive gliosis. Cell loss, as distinguished from cell involvement, was relatively slight in most segments of the central nervous system. The cerebral cortex appeared to be depleted more than the brain stem or spinal cord, but even in the cortex the decrease was not excessive. The degree of demyelination corresponded approximately to the magnitude of neuronal destruction. Loss of myelin substance was patchy and incomplete. No significant demyelination was perceived in the brain stem or spinal cord.

The peripheral nervous system (i.e., autonomic ganglia, retina, and posterior lobe of the pituitary body) was the site of microscopic changes equivalent to those described within the brain.

Examination of the remainder of the body showed a severe splenomegaly and hepatomegaly. In both these organs, as well as in lymph nodes and bone marrow, cells of reticulo-endothelial origin were distended with cytoplasmic vacuoles.

Case 3

S. G. was a male infant who was born prematurely by a breech delivery. There was no history of consanguinity and there were no other siblings. The infant was considered "backward" during the first 6 months of life, but not sufficiently so to provoke parental concern. Beyond that age the parents described an insidiously progressive apathy and disinterest in his environment. The child ceased to recognize his parents at the age of 1 year. Medical examination on admission revealed an obese, unresponsive child who appeared chronically ill. Cherry-red spots were noted in the fundi of both eyes. The child assumed a frog-like position with external rotation and semiflexion of the arms. The circumference of the head was increased (51.5 cm.), with an open anterior fontanelle. A marked hyperacusis was present. The patient failed to follow light or objects. The reflexes were universally hyperactive, though hypotonia was dominant at rest. Abdominal reflexes were present and bilaterally positive Babinski reflexes were obtained. Roentgenograms of the

skull demonstrated separation of the sutures. Roentgenograms of the chest showed pneumonic consolidation in the right upper lung.

His brief course in the hospital was marked by fever and dyspnea. Convulsive seizures developed. The child lapsed into a comatous state and expired shortly thereafter. Duration of the significant symptomatology was 14 months.

Necropsy Findings. At necropsy the brain weighed 1,300 gm. However, it appeared to be of normal volume. The leptomeninges were diffusely clouded, thickened, and edematous. This change was conspicuous in the dorsal sulci and the basilar aspects of the brain. The optic chiasm and related structures were largely obscured. Dissection of the cerebral hemispheres revealed a moderate internal hydrocephalus, symmetrically disposed, with no evidence of any obstructive lesions. The third and fourth ventricles shared in the process of dilatation. Within the subcortical white matter of both occipital and parietal lobes were a number of visually discrete zones of hemorrhagic softening. These foci were often flame-shaped and never measured more than 2 cm. in diameter. The remaining cerebral white matter was intact. No obvious demyelination was apparent, but a degree of grayish discoloration was visible within the confines of the occipital gyral white matter.

Microscopically, the meninges were thickened and hypercellular. The arachnoid trabecular cells were unusually prominent and distended with sudanophilic material. Numerous mobile, fat-filled macrophages and fibroblasts reduced the subarachnoid space to a thickened and hypercellular meshwork. The smaller blood vessels were increased in number, and capillary proliferation was seen irregularly in regions of more intense fibrosis.

In the cerebrum all levels revealed a plenary involvement of ganglion cells (Fig. 1). While all neurons participated to a variable extent in the process, the magnitude of cell loss was only moderate. Only in the cerebral cortex was a significant degree of depletion obvious. The subcortical regions of grossly hemorrhagic softening (Fig. 2) consisted microscopically of ischemic parenchymal necrosis with superimposed foci of hemorrhage. The neighboring blood vessels were entirely surrounded by activated microglia. The endothelial cells were usually hypertrophied and bulged into the vascular lumina (Fig. 3). Such cells were intensely sudanophilic. Many blood vessels contained clumps of fibrin adherent to distended endothelial cells. Sudanophilic substance could be found, also, within the confines of the fibrin masses. In the immediate vicinity of some of the areas of encephalomalacia, the smaller arteries were totally occluded by masses of fibrin contain-

ing neutral lipid (Fig. 4). Examination of vessels in structurally intact areas of the cerebral cortex also showed lipid-containing endothelium.

INTERMEDIATE COURSE

Case 4

M. D., a male infant, was hospitalized at the age of 13 months because of blindness, mental retardation, and generalized muscular hypotonia. There was no history of consanguinity. Pregnancy, delivery, and the first 6 months of life were normal. Listlessness and visual difficulty were noticed by the mother at about the seventh month. Admission examination divulged a typical bilateral macular degeneration with cherry-red spots. Pupils still responded to light stimulation. All extremities were markedly hypotonic. Deep tendon reflexes were present and of physiologic intensity. Clinical course was marked by numerous episodes of convulsions occasionally followed by bouts of decerebrate rigidity. Hyperacusis was prominently displayed. The child lapsed into a vegetative existence with recurrent pulmonic infections. Death occurred after 17 months of hospitalization and 23 months after onset of the disease.

Necropsy Findings. At necropsy the brain weighed 1,360 gm. The cerebral hemispheres were questionably enlarged and the dorsal gyri flattened. The sulcal convolutions were narrowed. The meninges were mildly thickened and milky in appearance. Dissection of the cerebrum, cerebellum, brain stem, and spinal cord revealed no notable lesions or alterations. Histologic studies confirmed the diagnosis and showed the typical neuronal changes in all segments of the peripheral and central nervous system, including the retina, autonomic ganglia, and posterior lobe of the pituitary gland.

PROTRACTED COURSE

Case 7

R. B., a female infant, was hospitalized because of developmental retardation and commencing blindness. The child was apparently healthy until the age of 4 months at which time the parents observed a change in behavior. Lethargy, failure to recognize objects or people, and weakness were described. On entry to the hospital, the patient was noted to be an apathetic, chronically ill, and poorly nourished, blind infant. The head was visibly enlarged (Fig. 5), measuring 53.6 cm. in circumference. The anterior fontanelle was widely patent. Macular degeneration was seen. A generalized hypotonia was evident. Reflexes were exaggerated. Roentgenographic examination of the skull showed open sutures. A pneumo-encephalogram demonstrated considerable enlargement of all ventricles (Fig. 6). An electro-encephalogram taken during the initial period of hospitalization was interpreted as normal. The patient remained oblivious to her environment throughout her stay. Her course was punctuated by frequent episodes of pneumonia. Shortly before death, the circumference of the head was 60.2 cm. as compared to a chest circumference of 49.5 cm. Cranial suture separation increased in accord with the progressive hydrocephalus. The child succumbed during an attack of pneumonia at the age of 36 months, and 32 months after the recognition of symptoms.

Necropsy Findings. At necropsy the much enlarged brain weighed 1,570 gm. (Fig. 7). Increase in volume was confined to the cerebral hemispheres, which were symmetric but abnormal in contour. The parietal and occipital lobes showed the greater degree of enlargement. The dorsal gyri were compressed and pallid. The leptomeninges were moderately opaque and thickened. Consecutive coronal sections of the cerebral hemispheres disclosed a thinning of the frontal gray matter in conjunction with a linear, depressed, necrotic zone situated within the cortex and running parallel to the leptomeninges. This zone was porous, edematous, and measured less than 1 mm. in width. The white matter of the frontal lobes was depressed, softer than normal, focally translucent, and occasionally assumed a semi-liquid consistency. The basal ganglia were obscured since no color distinction between gray and white matter could be established. In the parietal, temporal, and occipital lobes degeneration of the white matter was more pronounced (Fig. 8). No myelin was evident grossly. Within the parietal and occipital white matter, there were large, central areas of ragged cavitation. The arcuate fiber system was not spared. Many of the posterior parietal gyri were reduced to shells of cortical gray matter encompassing fluid-filled, uniloculate spaces (Fig. 10). The gyri of the parieto-occipital regions again demonstrated linear necrosis within the cortical gray matter and restricted to it (Fig. 9). In the caudal parietal gyri, two parallel linear zones of necrosis adhering to the tortuous configurations of the gyral surfaces were noted. On the other hand, dissection of the cerebellum showed only diffuse atrophy and increased tissue consistency. The brain stem and spinal cord grossly were unremarkable. Sections of the degenerated white matter showed total loss of the axonal and myelin constituents, which were replaced by proliferating astrocytes with numerous microglial elements filled with lipid (Fig. 11). The contained blood vessels were patent, but the endothelial cells invariably were enlarged and their cytoplasm was filled with delicate, polyloculated vacuoles. The cortex was almost totally depleted of neurons. An extensive astrocytosis and microgliosis persisted. Thin lamina of necrosis traversed the frontal and posterior parietal cortex longitudinally. The degenerated regions were devoid of any tissue structures and consisted only of detritus and collections of gitter cells.

Nature and Staining Characteristics of Intracellular Lipids

The characteristic histologic feature of amaurotic family idiocy is the infiltration of neuronal cytoplasm with a granular, refringent,

poorly stainable substance (Fig. 5). Klenk and others[8,9] have analyzed this adventitious substance, provisionally designated by them as "substance X." The compound is closely related to the cerebrosides and has been shown to contain neuraminic acid.

Tissues derived from 4 cases in the present series, in addition to routine fixation procedures, also were placed in chilled acetone for enzyme studies. The abnormal pre-lipid was mildly hematoxylinophilic. Following the silver impregnation techniques (e.g., Bielschowsky stain), a perceptible argyrophilia was noted. The substance stained pale pink with Sudan IV and inconsistently with Sudan black. The Schultz reaction for cholesterol was negative until such time as the pre-lipid was released into the interstitium. No reaction was obtained with the Smith-Dietrich procedure for phospholipids, the Feulgen stain, McManus' periodic acid-leukofuchsin method, metachromatic stains, or Gomori's alkaline phosphatase reaction. No esterase activity could be demonstrated with Gomori's technique. In sections derived from the central and peripheral nervous systems, ganglion cells displayed no difference in tinctorial or histochemical qualities.

Discussion

In Sachs' original communication[1] the brain was described as showing "great depth of all fissures and comparative simplicity of fissuration, particularly in the frontal lobes, and great exposure of the island of Reil." In his case, the weight of the brain was 1,000 gm. Subsequent reports have re-emphasized the narrowed gyri, broadened sulci, and secondary exposure of the deeper cerebral crevices. Meningeal opacity and thickening were prominently mentioned also. Dissection of the cerebral cortex invariably disclosed an increase in parenchymal density. Occasionally a pale, rubbery consistency was described. The clarity with which gray and white matter can be distinguished normally was obscured or lost. Some reports also alluded to a mild, uniformly distributed ventricular dilatation.

Macroscopic examination of the brain specimens of the so-called aborted cases, reported herein, conforms to the descriptive features outlined by others. Atrophy was of variable degree, but was evident in all instances. The cerebellum and brain stem shared in the atrophy. Histologic preparations in such cases revealed a ubiquitous degenerative process confined primarily to ganglion cells. An adventitious pre-lipid substance accumulated progressively within the neuronal cytoplasm, resulting in loss of the angular or pyramidal configuration of the cell. The Nissl bodies were diminished in number and the

residuum compressed about an eccentric nucleus. The neurofibrils usually were displaced peripherally by the abnormal cytoplasmic infiltrate, but remained intact until cell disintegration ensued. The integrity of the intracellular neurofibrils is closely related to neurocellular function. It would appear from this that nerve cells can be involved by the dystrophic process of this disease and still function, albeit at decreased efficiency. In many instances the history indicated an abrupt onset of the illness. The child usually was described as normal up to a certain point, at which time developmental progress was aborted and the pathognomonic features of the disease emerged. A saturation point evidently was reached beyond which central nervous system maturation could not proceed. One of the patients in the present report was given a routine physical examination at the age of 7 months. This apparently healthy infant showed no abnormal findings except for funduscopic evidence of early bilateral macular degeneration. Three months later, the first neurologic signs of amaurotic family idiocy were elicited (i.e., convulsions and arrested development). The circumstances indicated that neurons affected by the dystrophic process relinquish their basic functions only with reluctance and that a remarkable integrity of such functions is maintained until later in the course of this particular degenerative process. On this basis, it might be hazardous to establish a time of commencement of the disease. It is conceivable that the abnormal process exists at birth in a clinically inapparent fashion.

The extensive degree of atrophy, prominent surface fissuration, and increased tissue density, which exemplified cases from the first category, were not as prominent in specimens derived from cases with an intermediate course surviving up to 24 months. In one instance, the brain was described as being grossly normal in appearance. Microscopically, ganglionic degeneration was more pronounced, however. While no neuron within the nervous system was immune to the process of intracellular pre-lipid accumulation, some pattern of differential implication could be appreciated. The neurons comprising the olfactory bulbs and the dentate nucleus of the hippocampal cortex were but minimally involved and no perceptible cell loss was noted. The cerebral cortex and Purkinje and granular strata within the cerebellum were moderately depleted of neurons. The neurocellular components of the basal ganglia and thalamic nuclei showed a lesser degree of involvement and the magnitude of cell loss was appreciably smaller than in the cerebral or cerebellar cortex. Cells of the motor cranial nerve nuclei (e.g., hypoglossal, facial) demonstrated a more advanced stage

of ballooning than did the brain stem neurons serving the non-motor modalities. The inferior olivary nuclei, substantia nigra, and cerebellar nuclei (dentate, fastigial, and emboliform) were least affected. No significant cell loss could be seen in these nuclei and the extent of pre-lipid infiltration was irregular and minimal. Within the spinal cord the anterior horn cells were affected more appreciably than the cells comprising the lateral or posterior horns. Neurons of the retina, posterior root spinal cord ganglia, autonomic ganglia, and posterior lobe of the pituitary body showed an intermediate state of implication.

The relatively slight degree of abnormal cytoplasmic infiltration of the inferior olivary nuclei and olfactory bulbs, in contrast to that observed in the cerebral cortical gray matter, may be viewed from many directions. The olivary ganglion cells are normally pigmented, and the presence of this pigment may in some fashion retard the development or storage of the pre-lipid. Other formations of anatomically discrete pigmented neurons (viz., cerebellar nuclei and substantia nigra) show a similar resistance to the accumulative process. The extranuclear pigment of the substantia nigra, however, differs in chemical constitution from that found in the other nuclear structures. The physical presence, per se, of a cytoplasmic pigment would not seem to be an adequate hindrance to superimposed storage of still another substance, unless the pigment were indicative of an inherently variant, underlying, metabolic state. In any case, this speculation would not explain the minimal implication of the unpigmented neurons of the olfactory bulb.

Some observers have suggested that the unequal involvement within the central nervous system may be explained, or at least understood, on the basis of embryologic considerations. Reconsideration of the relative extent of implication within the cerebral cortex, in the light of ontogenetic sequence, discloses some interesting correlations. Within the supratentorial neuraxis, the olfactory bulbs and hippocampal cortex show the least degree of involvement in amaurotic family idiocy. Myelination of both these areas is complete at birth and, phylogenetically, they represent the most primitive aspects of the mammalian cortex (i.e., paleocortex and archicortex). The cerebral cortical areas of severest destruction, the frontal and parieto-occipital cortices, are the last fields to myelinate, the last to appear phylogenetically, and are concerned principally with higher correlative and associative functions. The pre-central and post-central convolutions become medullated at an intermediate stage, and correspondingly show an intermediate degree of involvement in the disease. There appears to

exist, then, a parallelism between ontogenetic status and the extent to which a cerebral region will be receptive to the basic disease process.

As the inexorable process of neurocellular dystrophy and degradation proceeds, the glial and mesodermal components of the nervous system participate in the disposal of the material previously accumulated in the ganglion cells. The abnormal stores of cytoplasmic pre-lipid are disseminated into the interstitium following total breakdown of the neuron. At this stage, the aberrant stored substance is only faintly sudanophilic. A prompt, often exuberant, microglial reaction ensues, resulting in phagocytosis of the released pre-lipid. Once within the cytoplasm of the activated microglial cell, the abnormal substance displays the staining capacities of neutral lipid. Where formerly it reacted poorly with the fat-soluble dyes, it now does so intensely. In addition, the material reacts positively for cholesterol using the Schultz technique.

There is a slow migration of lipid-distended microglia toward the perivascular spaces. Early in the course of the disease, the cortical blood vessels are encompassed by rings of such cells. Fat stains show the presence also of some free lipid adjacent to disintegrating microglial cells. A further migration within the perivascular spaces toward the leptomeninges follows, and large numbers of ballooned microglia are visible within the subarachnoid space. Arachnoid trabecular cells assume phagocytic capacity and pick up lipid from degenerating microglia. The subarachnoid space becomes increasingly cellular, particularly in the sulcal depths. Still later, diffuse fibrosis and increased vascularity supervene and are represented grossly as meningeal thickening and opacity.

In 6 cases of the present series one or more pneumo-encephalograms were performed, often after at least 18 months of illness. In 5 instances there was considerable distention of the lateral ventricles. The third and fourth ventricles evidenced enlargement, but no air was detectable in the subarachnoid spaces. Clinically, these 5 patients showed visible cranial enlargement. Mento-occipital circumferences were obtained at bimonthly intervals and invariably demonstrated an abnormal and progressive increase in measurement. The anterior and posterior fontanelles increased in lateral dimension as the illness and associated hydrocephalus persisted. Globus[5] and others recognized ventricular dilatation as a concomitant aspect of amaurotic family idiocy but failed to offer suggestions as to its causation.

In a case of gargoylism, a form of central nervous system dystrophy closely allied to amaurotic family idiocy, Russell[10] described an ob-

structive hydrocephalus and attributed the ventricular enlargement to the excessive subarachnoid fibrosis which was an outstanding feature of her case. Although the cerebral atrophy, no doubt, is a major factor in the etiology of the ventricular dilatation, the moderate leptomeningeal fibrous reaction suggests that it can be a contributing element in cerebrospinal fluid obstruction. Careful dissections in the region of the foramina of Luschka and Magendie, moreover, failed to disclose any local changes which might be held responsible. Similarly, no abnormalities of the intrinsic ventricular apertures or of the aqueduct of Sylvius were found.

Although it is presumed that most of the secondarily liberated lipid is carried passively to the subarachnoid space and there incorporated into the phagocytic, arachnoid, trabecular cells, a fraction of this material passes through the vascular wall and is absorbed by the endothelial cells. These cells, reverting to scavenger activity, are frequently hypertrophied and project into the lumen. The cytoplasm is granular and stains intensely with the Sudan IV technique. Fragmentation of the cell membrane eventually results in a release of the lipid granules into the vascular lumen. In one instance the endothelial rupture led to a superimposed fibrin deposition. Numerous cortical blood vessels were thrombosed, causing small zones of regional hemorrhagic encephalomalacia. These areas were well circumscribed and restricted to small segments of the cerebral cortex. Participation of endothelial cells in the disposition of lipid was noted only in the cerebrum (Figs. 3 and 4).

The average duration of life in cases of amaurotic family idiocy is a reflection of more than the natural progression of the degenerative process. The immediate cause of death is frequently a supervening secondary infection. All patients in the third category (children surviving longer than 2 years) were hospitalized shortly after establishment of the diagnosis and remained institutionalized until demise. Antibiotics and ancillary therapeutic agents undoubtedly played a major rôle in prolonging life.

There was nothing in the clinical features of the 4 extended cases, other than increased longevity, to differentiate them from the patients with the abbreviated disease. All showed macular degeneration and the distinctive retinal cherry-red spot. Extensive laboratory tests were performed on the sera, blood elements, and spinal fluid of the patients. No salutory changes were evident in all cases of the Tay-Sachs form of amaurotic family idiocy (regardless of duration of life) except for a mildly lowered serum phospholipid level, in contrast to the elevated levels noted in Niemann-Pick's disease. Electrophoretic studies of

serum carried out in only 4 children with the Tay-Sachs form of amaurotic family idiocy showed lowered gamma globulin and elevated alpha-2 globulin fractions. Similarly, cerebrospinal fluid proteins were moderately increased in the same patients but the gamma globulin component of spinal fluid was significantly depressed. The cephalin flocculation test on blood serum was uniformly positive. Other hepatic function studies were normal, and post-mortem examination of the liver failed to reveal any morphologic alterations capable of inducing changes in the cephalin flocculation test. Analysis of these findings will be reported elsewhere.

Gross inspection of the brain of each of the protracted cases disclosed a salient difference when compared with those of the other groups. In distinct contrast to the shrunken and atrophic specimens representative of the first and second categories of patients, the 4 cases of the third group had voluminous brains. The mean average weight was about 1,650 gm., considerably heavier than the anticipated brain weight for infants of this age. The cerebral hemispheres were enlarged and the gyri were compressed and flattened. The dorsal cortical surfaces were pale. The brain stem and cerebellum were characteristically atrophic, resulting in a pronounced disparity between the infratentorial and supratentorial regions. Coronal dissection of the cerebral hemispheres disclosed a gelatinous consistency of the white matter. The central degeneration was invariably most severe in the occipital white matter, becoming gradually less apparent in the rostral cerebrum. In regions of severest dissolution, irregular cavities were seen. Diffuse loss of myelin characterized the entire cerebral white matter, resulting in an indistinct demarcation of gray and white matter. The borders of the basal ganglia also were difficult to ascertain.

The macroscopic alterations in the 4 protracted cases of amaurotic family idiocy are reminiscent of those which characterize diffuse sclerosis (Schilder's disease). In both entities there is confluent cerebral demyelination commencing in the caudal poles with diminishing severity toward the frontal regions. Ragged central cavitation exemplifies both diseases. Some differences, however, also are evident. In diffuse sclerosis, the subcortical arcuate fibers generally are uninvolved by the process. In the protracted cases of amaurotic family idiocy no such immunity is seen, and in 2 cases the foci of most profound degeneration were centered within the gyral white matter and adjacent arcuate fiber system. Of greater importance is the fact that the cortical neurons are unaltered in diffuse sclerosis.

Microscopic preparations of the cortex offered some information

concerning the causation of this massive demyelination in protracted amaurotic family idiocy. The extent of ganglionic loss was far more devastating than in the cases with shorter duration. Only a small fraction of cortical neurons persisted. The cortex was thus reduced to a zone comprised almost exclusively of astrocytes, glial fibers, and residual microglia. Axonal stains failed to demonstrate any intact nerve fibers extending into the subjacent white matter. The white matter was totally replaced by dense, relatively cellular astrocytosis. No myelinated sheaths or axons could be demonstrated in the gliotic zone. The interstitium was intensely edematous. A moderate number of lipid-filled microglia also were seen. Gliosis of the cortex and white matter was of approximately the same magnitude and essentially contiguous, but the edema was restricted principally to the white matter. Thus, while the line of cortico-medullary distinction was grossly obscured in so far as color was concerned, some differentiation could be appreciated on the basis of texture and consistency. Numerous sections of degenerated white matter were stained for the presence of metachromatic granules, a characteristic feature of diffuse sclerosis.[11] None could be visualized.

Since these 4 cases of amaurotic family idiocy have displayed the coexistence of megalencephaly and massive deterioration of cerebral white matter, some doubt as to the diagnosis may arise in a disease generally exemplified by atrophy. There have been, however, isolated reports of other instances showing these presumably atypical features. Ostertag[12] reported the necropsy findings in a girl with amaurotic family idiocy whose relevant clinical symptoms commenced at 5 months. The patient died at the age of 38 months. The cranial circumference terminally was 52 cm. and the brain weighed 1,300 gm. Ostertag was impressed by the enlargement of the cerebral hemispheres in contrast to the shrinkage of cerebellum and brain stem. Gelatinous areas of degeneration of the cerebral white matter, most evident in the occipital lobes, was recorded. The ventricular system was dilated. Similarly, in Globus'[5] review, one of his cases was noted to show gelatinous degeneration of the cerebral white matter. Survival of the axonal appendage is dependent upon the integrity of the ganglion cell. It is not difficult, therefore, to accept orthograde axonal degeneration when one appreciates the overwhelming degree to which cortical ganglion cells are destroyed. In the later phases of this disease the massive gelatinous degeneration may be considered as an indication of the ubiquitous extent of the degradation.

The cerebral enlargement is more difficult to understand. In the

earlier aspects of the disease, the brain is eminently atrophic. As survival is extended, the process is reversed and megalencephaly emerges. This is visualized also in repeated pneumo-encephalograms. Ample sections from all cerebral regions confirm the degeneration of the neuronal elements, and disclose the potentiated microglial and astrocytic reaction which attends the breakdown of neurons, axons, and myelinated sheaths. Two divergent processes undoubtedly coexist. The number of ganglion cells, axons, and sheaths inexorably diminishes. The diffuse and continuing release of abnormal lipids into the interstitium visibly stimulates a glial reaction which is in excess of that associated with neuronal fragmentation from other causes. The enhanced glial productivity and contributory edema apparently outweigh the coincident structural losses, and eventuate in cerebral megalencephaly.

A curious form of cavitary encephalomalacia confined to the occipital and parietal cerebral cortex was recorded in the 4 protracted cases. The destroyed regions were linear in configuration, restricted to the zones of cortical gray matter approximately equidistant from the pia mater and subjacent white matter, and tended to run parallel to the surface of the cortical convolutions. Microscopically, there was total tissue dissolution and bordering glial reaction. No vascular abnormalities in the immediate vicinity of regions of cortical encephalomalacia could be found to account for the exquisitely localized degeneration. Numerous observers have noted a variation in dystrophic involvement of the various neuronal strata comprising the cerebral gray matter. Ostertag[12] was impressed by the more profound implication of cortical layer III in his case of amaurotic family idiocy. Vogt[13] carried out elaborate cyto-architectonic studies in a case of amaurotic family idiocy and concluded that there was a noticeable difference in the resistance to destruction between the various constituent laminae of the cerebral cortex. Bielschowsky[14] also has published a case in which a status spongiosus was noted in the cortical region corresponding to layer III. In the 4 presented cases exhibiting linear cortical destruction, the degree of architectural disorganization was too extensive to permit accurate localization to any particular lamina. One can go so far as to state that the regions of encephalomalacia were in the approximate vicinity of layers III and V.

The mammalian cortex is generally described in terms of six strata, three of which (layers II, III, and V) are referred to as the pyramids. Layer III, the lamina pyramidalis, is the intermediate cortical pyramidal layer. The resident cells of layer III do not differ appreciably

from the cellular components of layer II. There is nothing to indicate any unique properties to the cells of layer III or V which would account for the selective destruction of this lamina. Since the vast majority of cortical ganglion cells in all layers are destroyed during the terminal phases of the disease, the spongiform necrosis cannot be ascribed only to an augmented vulnerability of any particular cell stratum. Layers III and V, however, are also the location of a great concentration of unmyelinated corticopetal fibers as well as bands of myelinated axons running parallel to the cortical surface (the lines of Baillarger). Since we have suggested that the plenary destruction of cortical neurons was responsible for the extensive cavitary degeneration of the cerebral medulla, it would seem reasonable to anticipate an equivalent form of cavitary necrosis in those regions of the cerebral cortex containing a great concentration of myelinated axons. Layers III and V fulfill this requirement.

Summary

Ten cases of infantile amaurotic family idiocy have been described, 8 of which were necropsied. Two of the 8 cases examined pathologically fell within the category of Niemann-Pick's disease with central nervous system involvement. While there is an unequivocal biochemical distinction between the Niemann-Pick and Tay-Sachs variety of amaurotic family idiocy, no morphologic divergency within the central nervous system has been established. On this basis, the 2 cases of Niemann-Pick's disease were included in the series.

There is a fundamental clinical similarity among the 10 studied cases. All of the children were of Jewish ancestry and all developed normally for the first 4 to 7 months of life. Apathy, developmental regression, paresis, amaurosis, convulsive seizures, and inanition, in varying order, developed shortly thereafter. Three of the patients succumbed within 14 months of clinically determined illness. One case persisted for 23 months before death supervened. Four patients died between 24 and 42 months after onset of the disease. Two patients are still alive. Retrospective analysis of the 8 necropsied patients seemed to indicate that the increased longevity was due to careful nursing supervision rather than any inherently different quality of the disease. Some correspondence between duration of illness and necropsy findings was apparent. A moderate, undistinguishing, central nervous system atrophy was noted in the abbreviated cases succumbing within 14 months. This is in accord with previous pathologic descriptions of the disease entity. In the patient surviving for 23 months, atrophy of

the brain was no longer a prominent feature. Neuronal disintegration consequent to the dystrophic process was more advanced, however. Thrombi causing patchy cerebral encephalomalacia and meningeal fibrosis, which resulted in mild obstructive hydrocephalus, were seen. Such non-specific secondary phenomena were believed to be attributable to the vagaries of intracerebral transport of released lipid. The 4 cases characterized by prolongation of the illness (28 to 42 months) all displayed increased cranial circumference of a moderately severe magnitude; hydrocephalus was invariably demonstrable by pneumoencephalography. Necropsies in these 4 cases substantiated the ventricular dilatation and showed, in addition, a megalencephaly (1,520 to 1,800 gm.), indicating an actual increase in bulk of the cerebral hemispheres. A massive demyelination of the cerebral white matter and cystic degeneration mimicking the gross features of Schilder's disease were observed uniformly. A plenary destruction of the cerebral cortical neurons was noted in these 4 cases, leading to extensive axonal and myelin sheath degeneration, which probably was the basis for the macroscopically evident cystic degeneration of cerebral white matter.

It appeared, too, that an approximate parallelism existed between ontogenetic development and degree of involvement. Thus, the most recently developed cerebral areas seem to be the most susceptible to the disease process.

We wish to acknowledge the helpful cooperation of Dr. Abram Kanof and of Miss Florence Gilles. The photographs were prepared by Mr. Herbert A. Fischler.

REFERENCES

1. Sachs, B. On arrested cerebral development, with special reference to its cortical pathology. *J. Nerv. & Ment. Dis.*, 1887, **14**, 541–553.
2. Greenfield, J. G., and Nevin, S. Amaurotic family idiocy: study of a late infantile case. *Tr. Ophth. Soc. U. Kingdom*, 1933, **53**, 170–200.
3. Spielmeyer, W. Über eine besondere Form von familiärer amaurotischer Idiotie. *Neurol. Centralbl.*, 1906, **25**, 51–55.
4. Kufs, H. Über eine Spätform der amaurotischen Idiotie und ihre heredofamiliären Grundlagen. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1925, **95**, 169–188.
5. Globus, J. H. Amaurotic family idiocy. *J. Mt. Sinai Hosp.*, 1942, **9**, 451–503.
6. Rothstein, J. L., and Welt, S. Infantile amaurotic family idiocy. Its relation to Niemann-Pick disease and other disturbances of lipoid metabolism; report of two cases of Tay-Sachs disease with necropsy. *Am. J. Dis. Child.*, 1941, **62**, 801–843.
7. Ford, F. R. Diseases of the Nervous System in Infancy, Childhood and Adolescence. Charles C Thomas, Springfield, Ill., 1937, 953 pp.
8. Klenk, E. Beiträge zur Chemie der Lipoidosen. 3. Niemann-Picksche Krankheit und amaurotische Idiotie. *Ztschr. f. physiol. Chem.*, 1939–40, **262**, 128–143.

9. Sobotka, H. Chemical differentiation of Tay-Sachs disease and other lipoidoses. *J. Mt. Sinai Hosp.*, 1942, **9**, 795–798.

10. Russell, D. S. Observations on the pathology of hydrocephalus. *Medical Research Council, Special Report Series, No. 265*, His Majesty's Stationery Office, London, 1949, 138 pp.

11. Feigin, I. Diffuse cerebral sclerosis (metachromatic leuko-encephalopathy). *Am. J. Path.*, 1954, **30**, 715–737.

12. Ostertag, B. Entwicklungstörungen des Gehirns und zur Histologie und Pathogenese, besonders der degenerativen Markerkrankung bei amaurotischer Idiotie. *Arch. f. Psychiat.*, 1925, **75**, 355–391.

13. Vogt, M. Sur la destruction laminaire et aréale de l'écorce cérébrale dans un cas d'idiotie amaurotique. *Encéphale*, 1929, **24**, 509–527.

14. Bielschowsky, M. Zur Histopathologie und Pathogenese der amaurotischen Idiotie mit besonderer Berücksichtigung der zerebellaren Veränderungen. *J. f. Psychol. u. Neurol.*, 1920, **26**, 123–199.

15. Myers, B. Statistics concerning the height, weight and other measurements of fourteen hundred London children. *Brit. J. Child. Dis.*, 1926, **23**, 87–107.

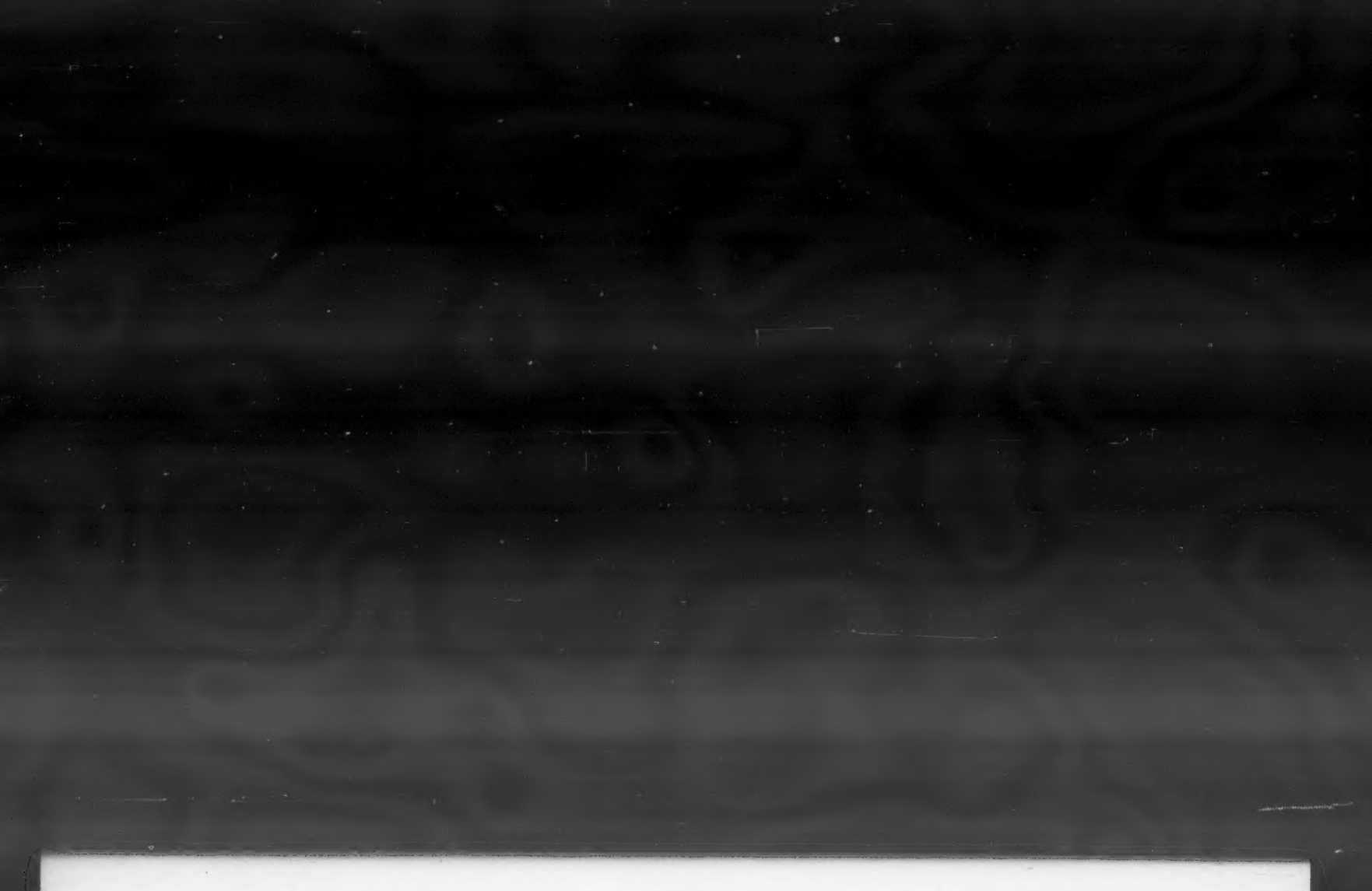
LEGENDS FOR FIGURES

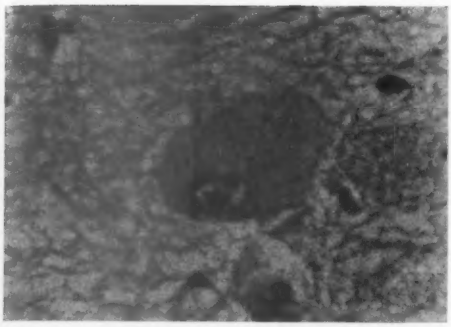
FIG. 1. (A) Cortical neuron from early case of amaurotic family idiocy showing ballooning, nuclear eccentricity, diminution in Nissl substance, and cytoplasmic infiltration with abnormal pre-lipid. Hematoxylin and eosin stain. × 650. (B) Silver impregnation stain of similar cell demonstrating peripheral displacement of neurofibrils. Davenport's stain. × 750.

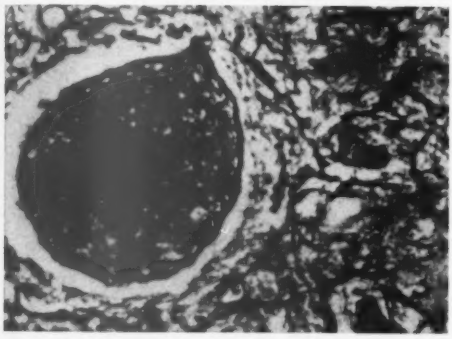
FIG. 2. Parietal cortex (case 4), showing discrete focus of subcortical hemorrhagic encephalomalacia.

FIG. 3. Blood vessel displaying endothelial enlargement within cerebral white matter in case of amaurotic family idiocy. The cytoplasm contains sudanophilic material. Hematoxylin and eosin stain. × 350.

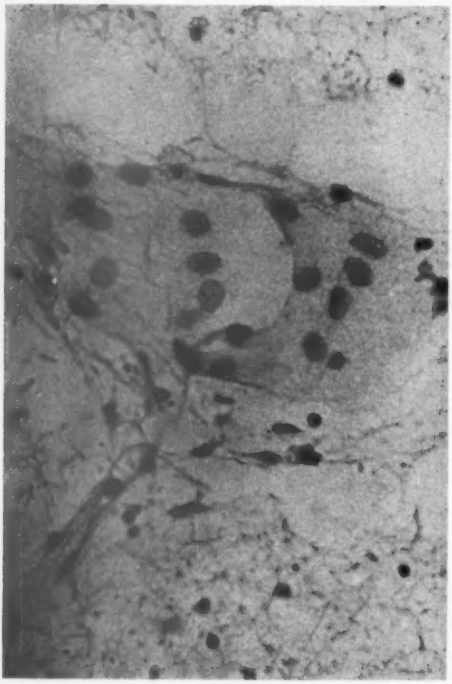
FIG. 4. Blood vessel of cerebral white matter (case 4) showing fibrinous occlusion. Some sudanophilic vacuoles are visible within the confines of the thrombus. Hematoxylin and eosin stain. × 350.

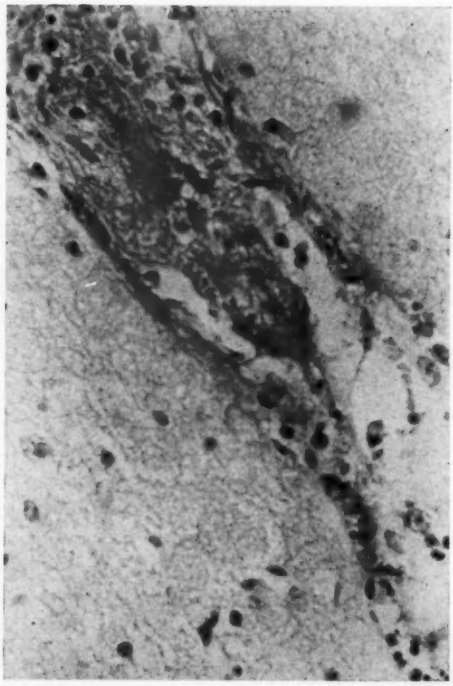






2



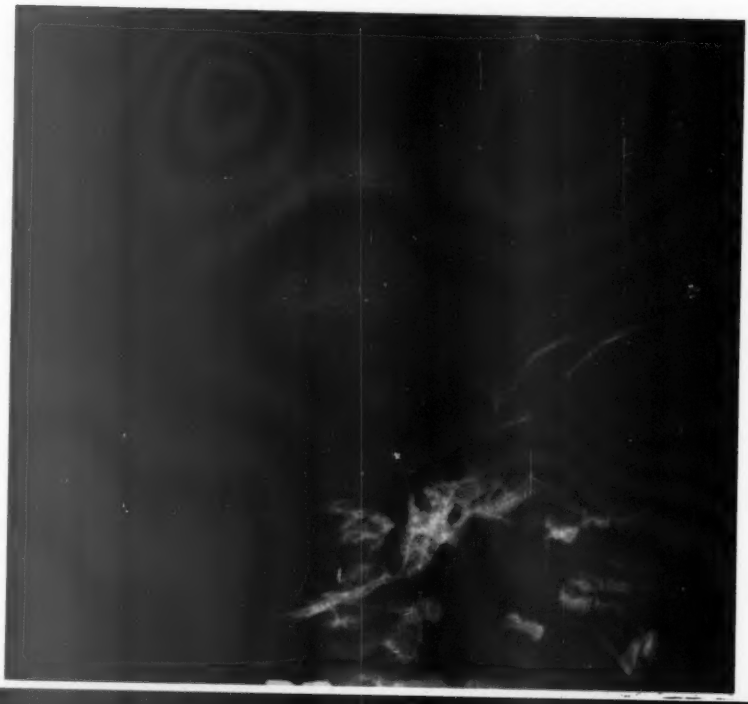


4

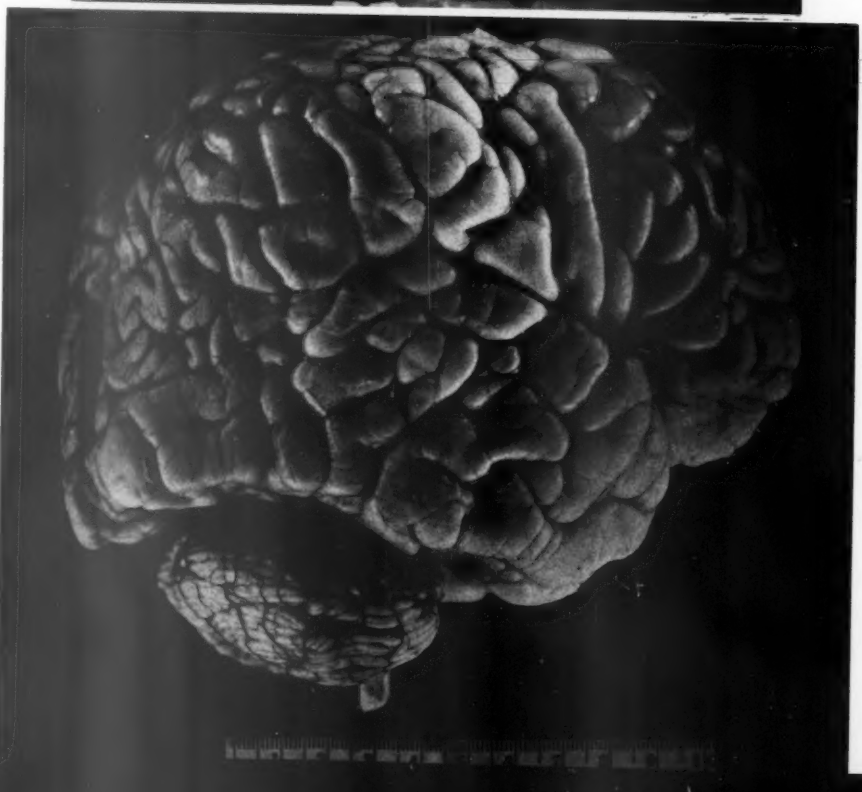
FIG. 5. Photograph of the patient known as case 7 showing perceptible increase in cranial circumference, the head measuring 60.2 cm. The normal circumference for this age is 47.7 cm.

FIG. 6. Pneumo-encephalogram, lateral view, of the same patient demonstrating uniform dilatation of lateral ventricles. This roentgenogram was taken a short time after the clinical photograph (Fig. 5).

FIG. 7. Appearance of undissected brain of case 7, showing the megalencephaly, associated with moderate flattening and pallor of the dorsal cerebral gyri. The cerebellum and brain stem do not participate in the process of enlargement.



6



7

FIG. 8. Coronal section of brain (case 7), showing massive demyelination and cystic degeneration of parietal white matter. Moderate ventricular dilatation.

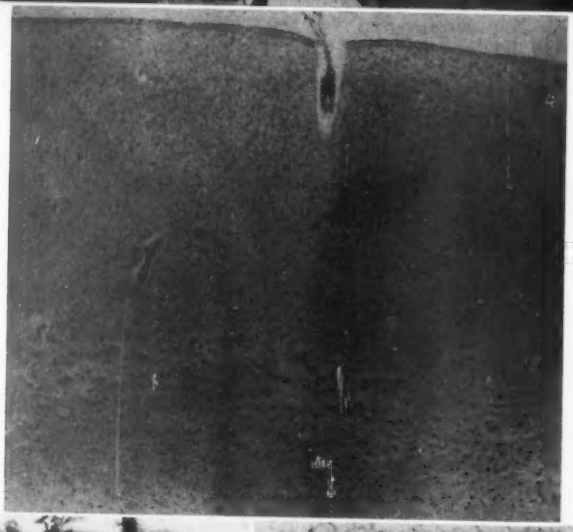
FIG. 9. Low-power photomicrograph of parietal cortex (case 7), showing linear encephalomalacia running parallel to the gyral surface. Hematoxylin and eosin stain. × 35.

FIG. 10. Superior parietal gyrus (case 7), revealing total cystic degeneration of subcortical white matter with destruction of arcuate fibers.

FIG. 11. Central parietal white matter near area of cystic degeneration (case 7). There is a total loss of axons and myelin sheaths with replacing cellular astrocytosis. Mallory's phosphotungstic acid hematoxylin stain. × 330.

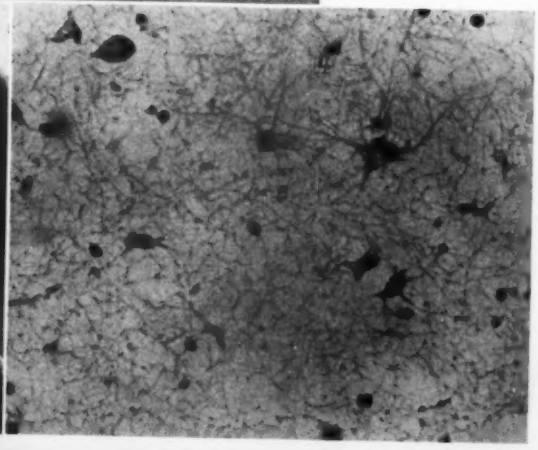
10

8



9

10



11

SARCOMA ARISING IN GLIOBLASTOMA OF THE BRAIN*

IRWIN H. FEIGIN, M.D., and SIDNEY W. GROSS, M.D.

From the Pathology and Neurology Departments of the Mount Sinai Hospital and the College of Physicians and Surgeons, Columbia University (Dr. Feigin) and the Neurosurgery Department of the Mount Sinai Hospital (Dr. Gross), New York, N.Y.

This paper will describe three primary, malignant brain tumors, each of which was composed of two dissimilar malignant tissues. One, of glial origin, was essentially like that in glioblastoma multiforme. The other, mesenchymal in origin and character, and resembling a spindle cell fibrosarcoma, is believed to have originated secondarily in the walls of the hyperplastic blood vessels seen so commonly in glioblastoma.

Tumors of this sort were described in the older literature some 60 years ago and the term gliosarcoma, analogous to the term carcinosarcoma, was restricted to such tumors by some investigators of that period. Subsequently, the existence of such tumors was discounted, the term gliosarcoma being applied to tumors which resembled sarcoma, but were thought to be nonetheless of glial origin. More recently, the term has been almost completely discarded as the glial origin of most of these tumors has been firmly established, and most would now be designated as glioblastoma multiforme. It is considered unwise to attempt to resurrect the term gliosarcoma for use in its original sense, because of the confusion which might result.

REPORT OF CASES

Case 1

The patient (P63993) was a white woman, 69 years old, with hypertension, who was well until about 2 months before death, when she began to drop objects from her left hand. A few weeks later, progressive paresis of the left foot became apparent and she became bedridden about 5 weeks prior to death. On examination, the patient was alert but disoriented and appeared severely ill. There was slight nuchal rigidity and a severe left hemiplegia with a positive Babinski sign on the left. She was incontinent. She was thought to have a metastatic neoplasm of the brain, but a thorough investigation of the gastrointestinal and genitourinary tracts, chest, and skull showed no evidence of abnormality. The electro-encephalogram showed severe slowing on the entire right side. A ventriculogram showed marked dilatation of the lateral ventricles, with the entire ventricular system deviated to the left. The third ventricle was markedly pushed over to the left, and more so superiorly than inferiorly. The posterior portion of the right lateral ventricle was deviated downward. Immediately following ventriculography, a right parietal osteoplastic flap was turned

* Presented in part at the Fifty-first Annual Meeting of the American Association of Pathologists and Bacteriologists, Philadelphia, April 9, 1954.

Received for publication, October 29, 1954.

down. A mass was found in the inferior parietal region attached to the dura over an area about 15 mm. in diameter. The mass was first thought to be a meningioma. A complete removal of the tumor was performed. The patient's condition remained poor, without a return of consciousness, and she expired 9 days postoperatively. Permission for necropsy was not obtained.

The surgical specimen consisted of a roughly globular mass of firm, yellow, gray, and tan tissue, measuring approximately 45 mm. in diameter and weighing approximately 41 gm. It was attached at one aspect to a small fragment of a dense collagenous membrane, 20 by 30 mm. in size, which resembled dura. The tumor mass was irregularly lobulated and well circumscribed, appearing encapsulated in some areas but not in others. On section, the tumor was quite firm and varied in appearance, being white in some areas, yellow, gray, or tan in other zones, and it contained foci of hemorrhage.

On section, two discrete though intermingling types of neoplastic tissue were apparent in hematoxylin and eosin preparations (Fig. 1). In one zone, the cells were moderate to small in size, with round, elongated or irregular, deeply chromatic nuclei. The cytoplasm of most of these cells could not be distinguished, but a few possessed a somewhat hyaline, eosinophilic cytoplasm, the margins of which were not sharply defined. The cells varied considerably in size, shape, and staining characteristics, and fairly numerous mitotic figures could be seen. In places, large, bizarre, giant forms were noted. The cells were arranged without order in a coarsely reticulated, eosinophilic matrix. These cells infiltrated individually into the adjacent normal cerebral parenchyma. The walls of some of the blood vessels in this area showed hypertrophy and hyperplasia of the endothelial and adventitial cells, and these changes were marked in places. The cells of some of these markedly hyperplastic vessels could be seen to pass outward, infiltrating the surrounding tissues, in which circumstance they were indistinguishable from the second neoplastic tissue to be described. This first tissue was considered to be of glial origin, in the nature of a glioblastoma multiforme.

The cells of the second tissue were considerably larger, with moderately chromatic, reticulated, ovoid, or elongated nuclei, between which ran coarse, deeply stained eosinophilic fibers. These cells showed moderate to marked variability in size, shape, and staining characteristics, and a moderate number of mitotic figures. The cells were generally arranged in long parallel rows which tended to interweave. In places they formed columns, on each side of which the more irregular glioblastomatous tissue was present. This tissue also assumed the form, on section, of circular islands of varying size, surrounded by glio-

blastomatous tissue (Fig. 2). The smaller islands could not be distinguished from the hyperplastic blood vessels. This tissue was considered to be a spindle cell fibrosarcoma arising from blood vessel walls. Large zones of necrosis were present, apparently involving both tissues.

In phosphotungstic acid-hematoxylin preparations,[1] the fine fibers of the gliomatous tissue were stained blue. The coarse fibers of the sarcomatous tissue and portions of the blood vessel walls were stained tan. With the azocarmine technique,[2] the fine fibers of the gliomatous tissue were stained red; the coarse fibers of the sarcomatous tissue, blue. The Wilder silver method for reticulin[3] stained some of the fibers of the sarcomatous tissue.

Summary. A 69-year-old woman died within 2 months of the onset of symptoms of a brain tumor lying superficially over the right inferior parietal area. The tumor was circumscribed, firm, and generally gray or white. Section revealed it to be composed of a mixture of glioblastomatous and sarcomatous tissues. The latter was thought to have arisen from the walls of the blood vessels of the former.

Case 2

The patient (A15698) was a white woman, 50 years of age, who was awakened suddenly by a throbbing, severe pain in the back of her head, 3 months prior to death. The headache persisted, and a few days later there was a syncopal attack. On examination, the only changes were bilateral, severe papilledema with hemorrhages. Lumbar puncture revealed xanthochromic fluid under a pressure of 240 mm., with 150 mg. per cent of protein. An electro-encephalogram showed moderate focal slowing in the right parietal region. Angiography was attempted but visualization of the carotid vessel was not obtained. Ventriculogram showed a defect in the region of the atrium of the right lateral ventricle, and a displacement of the posterior part of the third ventricle. The findings were interpreted as indicating a tumor arising deep in the region of the splenium of the corpus callosum. Immediately following ventriculography, a large, lateral, osteoplastic flap was turned down. The dura was under a marked increase in tension. On opening the dura, there was no evidence of a surface lesion. A biopsy specimen was obtained with a needle. The patient's condition remained more or less stationary and she was discharged to a nursing home after 6 weeks in the hospital. Two weeks later, she again complained of severe headache and was re-admitted. She was drowsy, disoriented, and bilateral papilledema was present. There was complete hemiplegia and a sensory defect on the left. The patient's condition deteriorated, and she expired 17 days after her second admission.

The biopsy specimen obtained by needle was not considered diagnostic. It included a group of cells which were thought to be mesenchymal, and the suggestion was made that this might represent a vascular reaction to a tumor, such as the glioblastoma.

Permission for necropsy was limited to an examination of the brain, which weighed 1,600 gm. (Fig. 4). There was a herniation of brain

substance at the operative site, with dural adhesions. The right cerebral hemisphere was larger than the left. The gyri were flattened on both sides, with concomitant narrowing of the sulci. The anterior portion of the right cingulate gyrus was herniated beneath the falx for a distance of 6 mm. The right uncus was considerably more protuberant than the left, although a distinct tentorial groove was not present. The cerebellum and brain stem were normal externally. Section of the cerebrum revealed a tumor nodule approximately 5 cm. in diameter, filling the midportion of the right lateral ventricle and extending into the tissues of the adjacent hemisphere. It was sharply circumscribed, and appeared encapsulated in areas. It was firm to hard, gray white, and presented a finely fibrillar, multilobulated, somewhat whorled appearance in most areas. Zones of hemorrhage and soft yellow zones of necrosis were present. There were a few small cysts with a gelatinous fluid. The surrounding brain was markedly edematous. The midline structures were pushed to the left for distances of 1 to 4 cm. The septum and corpus callosum were rotated. The lateral ventricles were both narrowed, except for some dilatation of the temporal horn on the right. The third ventricle was narrowed, its superior portion being deviated to the left. The aqueduct was laterally compressed. A small linear vertical hemorrhage was present in the midline of the tegmentum of the midbrain. The cerebellum was normal. The gross impression was that the tumor was a meningioma.

Microscopically, tissues of varying character were observed, complexly intermingled. One was a highly cellular tissue composed generally of small, dark cells with indefinite cytoplasmic outlines and small, round or ovoid, relatively irregular, deeply staining nuclei. These cells were diffusely distributed in a reticulated matrix. Many of the cells were large and had large, bizarre, deformed nuclei; and many mitotic figures were observed. A few of the cells contained a hyaline, eosinophilic cytoplasm with somewhat stellate outlines. These cells had infiltrated individually into the adjacent cerebral parenchyma. Large zones of necrosis were present as well as a few small protein-filled cysts. This tissue was considered glioblastomatous.

A second tissue type was composed of elongated cells with indefinite cytoplasmic outlines and elongated or fusiform, moderately stained nuclei. These nuclei were irregular in size and shape and were frequently large and misshapen. They were arranged in long parallel rows, between which coarse eosinophilic fibers were recognized. A variant of this tissue, found frequently about blood vessels, was less cellular, some of the cells being smaller and somewhat shrunken, but

characterized particularly by the presence of a slightly basophilic, mucoid, intercellular substance. These tissues were considered mesenchymal and sarcomatous.

The blood vessels of the glioblastomatous areas revealed the characteristic hypertrophy and hyperplasia of endothelial and adventitial cells to varying degrees. In places, this was marked. In some such cases the cells showed a striking variability and disorganization, with occasional bizarre, giant forms (Fig. 3). In many instances, a continuity could be traced between the tissues of the vessel wall and the tissues forming the interlacing bands of the sarcoma. Larger islands of sarcomatous tissue surrounded by glioblastomatous tissue could be found also.

The fibers of the glioblastomatous tissue generally stained blue with phosphotungstic acid-hematoxylin,[1] red with the azocarmine technique,[2] and were unstained with the Wilder silver stain for reticulin.[3] The fibers of the sarcomatous tissue generally stained tan with phosphotungstic acid-hematoxylin, blue with the azocarmine technique, and many were positive with the Wilder reticulin method. The perivascular mucoid material was slightly metachromatic with toluidine blue in an aqueous media, this property being lost when the stained paraffin sections were passed through alcohol.

Summary. A 50-year-old woman died within 3 months of the onset of symptoms of a brain tumor within the midportion of the right lateral ventricle and the tissues of the adjacent hemisphere. The tumor was circumscribed, gray, and very firm. On section, it consisted of a complex admixture of glioblastomatous and sarcomatous tissue. The latter was thought to have arisen from the walls of the blood vessels of the former.

Case 3

A man (A15445), 24 years old, began to complain of constant throbbing headache approximately 7 months prior to death. This was followed by impairment of vision on looking to the left. His headaches became increasingly more severe and were accompanied by vomiting. On admission, the patient was alert and cooperative but shortly became drowsy. Examination revealed a left homonymous hemianopsia and bilateral choked disks. Reflexes, sensation, and motor power were normal. A right percutaneous carotid angiogram disclosed marked elevation of the middle cerebral artery. The venogram showed depression and straightening of the internal cerebral vein. A right temporal craniotomy disclosed a large mass at the temporoparietal junction, just above the sylvian fissure. Subtotal removal of the tumor was performed. The patient improved rapidly and was discharged from the hospital 2 weeks later. He received a course of high voltage x-ray therapy and remained well for about 5 months when he complained of severe low-back pain and difficulty in urination. He began to vomit again and had twitching of the left side of the body. On admission to the hospital, there was a generalized convulsive seizure with loss of consciousness. After regaining consciousness he was disoriented. There were inter-

mittent clonic movements of the right upper and lower extremities. Sensation seemed to be disturbed in both lower extremities. The course was progressively downhill, and he expired 12 days after the second admission.

The surgical specimen, weighing 11 gm., included fragments of a very cellular neoplasm (Fig. 5). The cytoplasm of the neoplastic cells could not be distinguished. The nuclei were round, ovoid, or elongated, and generally deeply chromatic. The cells varied greatly in appearance and numerous mitotic figures were present. The cells were packed in masses of viable tissue, generally about blood vessels, with large zones of necrotic débris intervening, so that an almost papillary structure was formed. The cells at the margins were even more closely packed as if by pressure from the necrotic zones. In most areas no orderly cellular arrangement could be recognized within these cell masses. In a few, the cells stained more lightly, were consistently ovoid or elongated, and were arranged in parallel rows. Some of these zones were close to the blood vessel walls. In some of these areas the azocarmine stain disclosed a few blue-staining fibers, which were generally absent elsewhere. A diagnosis of sarcoma was offered.

At necropsy, the viscera were congested, and there was bronchopneumonia of both lower lobes. The brain weighed 1,460 gm. There was a defect in the dura at the operative site, 9 by 7 cm., in the right temporoparietal area. This defect was filled by a firm, fibrotic mass, adherent to the underlying brain and projecting approximately 1 cm. above it. A similar zone of dural adhesion to brain, 3 by 2 cm., was present on the medial surface of the right occipital lobe. The gyri and sulci were normal, and there was no evidence of cingulate or uncal herniation. Section of the brain revealed a mass of abnormal tissue extending from the operative dural defect, posteriorly and medially, to reach the zone of dural adhesion at the medial surface of the occipital lobe. These tissues varied from soft to very firm, the former areas appearing necrotic. The color varied from gray-pink to yellow and brown. Small cysts were present. The neoplastic tissue extended to the ependymal surface of the ventricle. The leptomeninges of the spinal cord contained small, circumscribed, thickened areas of opacity. Distinct neoplastic tissue was not recognized on the cord.

Sections of the necropsy material revealed much of the tumor to be necrotic. The viable portion varied in appearance. Some zones consisted of elongated cells with indefinite cytoplasmic outlines and elongated, moderately chromatic nuclei. These cells were arranged in long parallel rows with coarse, eosinophilic fibers between them. There was moderate variability among these cells, but mitotic figures were not observed. Clumps of brown pigment and a few mast cells were present.

In comparison with the surgical specimen of about 6 months earlier, the tissue was far less anaplastic. In adjacent areas the tissue was less cellular, but the cells were considerably more variable in appearance. Occasional large, bizarre forms were present. Large quantities of hyalinized eosinophilic material were present between these cells. This hyaline material and the coarse fibers in the more cellular zones stained blue with the azocarmine method[2] and tan with phosphotungstic acid-hematoxylin.[1] These tissues were considered sarcomatous.

In one small zone a distinctly different type of tissue was encountered (Fig. 6). In this area small, dark cells, varying greatly in size, shape, and staining characteristics, were scattered without order in a reticular matrix. Some were large and bizarre. The nuclei were round or irregular and deeply chromatic. At the margin these cells were seen infiltrating the normal parenchyma individually for variable distances. The margin with the sarcomatous tissue was more distinct. No blue-staining fibers with the azocarmine technique were seen in this area, except in the walls of the blood vessels, many of which were hyperplastic. On phosphotungstic acid-hematoxylin stain, a few small clusters of blue-staining fibers were noted. This small area was considered glioblastomatous.

Summary. A 24-year-old man died about 7 months after the onset of symptoms referable to a tumor in the right temporoparieto-occipital region. He had been treated by subtotal removal and x-ray therapy. The surgical specimen was interpreted as a highly malignant neoplasm, probably a sarcoma. At necropsy, the bulk of the visible tumor was considered to be a sarcoma, less anaplastic than the original surgical specimen, with considerable hyalinization. A small area of tissue typical of glioblastoma multiforme was present also.

Discussion

Criteria for Diagnosis

The tumors described are of interest in that they were composed of an admixture of two dissimilar neoplastic tissues. One tissue resembled glioblastoma multiforme, containing characteristic small, dark cells diffusely distributed in an eosinophilic fibrillar matrix (Figs. 1 and 6). These fibers resembled glial fibers in that they were stained blue with the phosphotungstic acid-hematoxylin technique,[1] red with the azocarmine technique,[2] and were generally unstained in Wilder's silver preparations for reticulin.[3] The other tissue resembled fibrosarcoma, being composed of large cells with elongated or fusiform, moderately chromatic nuclei, often arranged in parallel rows along with deeply eosino-

philic coarse fibers (Figs. 1 and 5). These fibers resembled connective tissue fibers in that they were stained tan by the phosphotungstic acid technique, blue by the azocarmine method, and many were deeply stained in Wilder's silver preparations for reticulin. The two tissues were intimately interwoven in many areas. Each tissue was histologically malignant, as evidenced by mitotic figures, high cellularity, and significant atypism and variability, and zones of necrosis were present involving both tissues. The tumor grew rapidly, destroyed the normal parenchyma, and caused death in less than 1 year.

In the glioblastomatous areas, the marked hyperplasia and hypertrophy of the cells of the vessel walls, characteristic of that tumor,[4] were clearly evident. In some vessels these changes were particularly marked and were associated with an appreciable atypism, cellular variability, mitotic figures, and disorganization, with occasional distorted, highly bizarre cells, so that the appearance suggested that a neoplastic transition had occurred (Fig. 3). In some areas, these cells extended outward from the wall to form masses of sarcomatous tissue. The clearly sarcomatous, infiltrative tissue often formed rounded nodules and masses encompassed by strands of glioblastomatous tissue, as if the sarcomatous tissue had arisen from a structure located within the existing glioblastoma (Fig. 2). It is concluded that the sarcomatous tissue did develop as a result of neoplastic change in hyperplastic blood vessel walls, the vessels themselves being present as part of the reaction to the presence of the malignant glioma.

This interpretation is made with the knowledge of some diagnostic pitfalls which tend to render a distinction between the two types more difficult. The presence of elongated spindle-shaped cells is not in itself sufficient to warrant a diagnosis of sarcoma. It is well known that in some locations, particularly in the brain stem, glial cells may assume this shape. The elongated astrocytes which form the "piloid" astrocytomas of this region, and the less mature "spongioblasts" of the spongioblastoma polare, are typical examples. The term central neurinoma applied to some of these tumors reflects the similarity to the tumors of the peripheral nerve sheaths, with their abundant collagen fibers. These gliomas of the central nervous system may be distinguished by showing that the fibers among the cells are of glial, not connective tissue, character by the staining techniques utilized in the present study.

At the same time, the mere presence of connective tissue fibers within the tumor is not in itself sufficient to warrant a diagnosis of sarcoma. Many gliomas infiltrate the subarachnoid space and there evoke a response in the arachnoid cells of the leptomeninges which

will be associated with a proliferation of connective tissue fibers. Neoplastic glial cells will then lie in relation to connective tissue fibers as well as glial fibers. It usually is possible to recognize in the section that this process is taking place in the subarachnoid space. Such areas are characterized by marked disorganization rather than presenting the orderly arrangement of cells in parallel rows seen in sarcoma and in the sarcomatous portion of the tumors being described.

A Note on Nomenclature

Although in recent years the existence of such mixed tumors of the brain has received little attention, this possibility has been the subject of considerable study in the older literature. As early as 1895, Stroebe[5] referred to tumors of glial origin within which a sarcomatous growth derived from blood vessel walls could be distinguished. He urged that the much used term gliosarcoma should be restricted to such tumors containing both elements. It would seem that others experienced greater difficulty in distinguishing these tissues or differed in their interpretation. Hildebrandt[6] and even Ewing[7] subsequently used the term gliosarcoma to refer to tumors which resembled sarcomas but were believed to be of glial origin. The terms glioblastic sarcoma[8] and glioma sarcomatosum[9] appear to be of similar significance. Wohlwill[10] expressed some doubt as to the existence of true mixed tumors as described by Stroebe, although he mentioned what he thought to be the simultaneous apposite occurrence of a glioma and a sarcoma. Globus and Strauss[11] implied that Stroebe had misinterpreted as neoplastic the hyperplastic vascular reaction seen so commonly in gliomas, particularly in glioblastoma multiforme. In any case, the term gliosarcoma was used generally for many years for some of the malignant gliomas.[7] Largely through the studies of Globus and Strauss and of Bailey and Cushing,[12] such tumors have now been firmly established as of glial (ectodermal) origin, so that the term sarcoma would be inappropriate, and most of these tumors would now be classified as gliobastoma multiforme.

While one might be tempted to resurrect the term gliosarcoma for these tumors in the sense originally described by Stroebe and as an analogue to carcinosarcoma, such an attempt would undoubtedly lead to greater confusion and is considered inadvisable.

A Historical Review

In 1905, Babes[13] described a large tumor of the right temporal lobe and the midline basal structures of a 33-year-old woman who died after an illness of at least 3 months. Microscopically, the bulk of the

tumor was considered to be a sarcoma arising from the walls of blood vessels. However, proliferating neuroglial elements were seen, particularly in zones which had become necrotic. Although it was stated that it was not absolutely certain that the glial elements participated in tumor formation, this was strongly suggested by the author.

In 1910, Merzbacher and Uyeda[14] described tumor masses in the right cerebral hemisphere of a 25-year-old man, who died after an illness of 2½ years. The tumor consisted of two adjacent neoplastic masses easily distinguished on gross examination, the point of junction being sharply defined. Microscopically, one tumor was described as a sarcoma, the other as a glioma, and some interweaving of the tissues at the line of junction could be recognized. The sarcoma was traced to the leptomeninges, and the authors expressed the opinion that the glioma had arisen secondarily as a reaction to the irritation induced by the sarcoma. Subsequently, Wohlwill[10] described tumor masses in the right frontal lobe of a 55-year-old woman who had experienced convulsive episodes since the age of 9, and had shown more definitive clinical manifestations of brain tumor for 3 years. The tumor consisted of two discrete masses separated by a clearly recognizable band of white matter. Microscopically, it was recognized that one tumor was a sarcoma, the other a glioma, and while the tumors were apposed to each other, there was only one small microscopic area of mutual infiltration. The author suggested that the two tumors were of independent origin.

In 1934, Bailey and Ley[15] reported the case of a 6-year-old boy who had had one convulsive episode at 8 months of age, and developed definitive signs of brain tumor 4 months prior to death. At two operative procedures, large masses of tissue recognized as perithelial sarcoma were removed, but, in addition, some of the tissues removed at the second operation appeared to represent an astroblastoma. At necropsy, a large tumor mass in the right temporal lobe proved to be largely astroblastomatous, with a small mass of residual sarcomatous tissue in one area. The sarcomatous area was grossly separated from the glioma by a band of compressed brain. The authors expressed the opinion that the glioma was primary, and that the sarcoma arose at a later date from blood vessels. This may be the same case as that designated case 85 in the comprehensive study of brain tumors of children by Bailey, Buchanan, and Bucy[16] some years later.

In 1949, French[17] described a brain tumor in the right fronto-temporo-parietal area of an 18-year-old girl, who died 27 months after the onset of symptoms. Surgical material removed at the first oper-

ation, about 24 months before death, was typical of an astroblastoma. The biopsy material at a second operation, about 20 months before death, contained fragments of two different types of tissue. On section, one tissue was astroblastomatous while the other was characteristic of a perithelial sarcoma. No fragments containing both tissue types were seen. At necropsy, the remaining neoplastic tissue examined was all astroblastomatous, but much of the tumor had been inadvertently lost. The author expressed the opinion that he was dealing with two dissimilar tumors which arose independently in the same portion of the brain.

An additional report of the simultaneous occurrence of glioma and sarcoma is that by Nichols and Wagner[18] of a 69-year-old woman with a sarcoma in one cerebral hemisphere and a spongioblastoma polare in the other (case 7).

It would appear that the tumors described by Babes,[13] Bailey and Ley,[15] and French[17] may be similar to those described in this paper. The tumors described by Merzbacher and Uyeda[14] and Wohlwill[10] are less likely to be of this nature, while the situation described by Nichols and Wagner[18] is almost certainly unrelated.

The Significance of the Observations

These tumors are of limited significance in a clinical sense. Glioblastoma multiforme shows a poor response to any known therapeutic procedure, and the prognosis is not altered by the presence or absence of a superimposed sarcomatous change in its blood vessels. The sarcomatous tissue is very much harder than the glioblastomatous tissue, and the neurosurgeon who anticipates a glioblastoma might be misled by the consistency of such tumor into thinking that he is dealing with a benign meningioma. This would be particularly true when the tumor presents itself on the surface of the brain and appears to form a circumscribed mass, as in case 1 of this report. Glioblastoma also has been reported to assume such apparently circumscribed surface forms.[19]

These tumors may prove to be of greater significance because of the contribution their study might make to our knowledge of the pathogenesis of neoplasms. The relationship of one neoplastic tissue to the induction of neoplastic change in another tissue is of fundamental interest. This problem has been studied statistically by comparing the observed incidence of neoplastic change in more than one tissue with the incidence calculated on the supposition of unrelated coincidence. The most recent such study, that of Watson,[20] concludes

that the incidence of true multiple tumors is statistically coincidental and that the data did not support the thesis of either a constitutional tendency to develop a second cancer or of the development of an immunity to such second cancer. Earlier studies, such as those of Bugher,[21] Slaughter,[22] and Warren and Ehrenreich,[23] suggest that the observed incidence is significantly greater than that expected by chance.

More direct information has been sought in studies on tumors of other organs analogous to those being described in the brain. Such tumors, the carcinosarcomas, are believed by some to be composed of mixed malignant epithelial and mesenchymal tissues. These tumors have been most difficult to analyze, and their very existence is a matter of controversy. Saphir and Vass[24] reviewed 153 previously reported cases of this type, and accepted only 3 or 4. Willis[25] was reluctant to accept any human case of this type, although he believes that some animal tumors might be of this character. Harvey and Hamilton,[26] on the other hand, aware of all diagnostic pitfalls, are convinced of their existence, and reported 6 cases of their own.

In animals, tumors of this type have been induced by carcinogenic agents.[27] Experimental data have been interpreted as indicating the malignant transformation of the connective tissue stroma of transplanted animal tumors.[28-31] In some of these reports it is conceded that the true sarcomatous nature of the morphologically sarcoma-like neoplastic tissue is not conclusively demonstrated.[31] Others are convinced of the accuracy of such designation[29,30] and one report offers evidence of this based on the cultural growth characteristics of the neoplastic tissue and its inhibitory effect on the growth of fibroblasts *in vitro*, a property characteristic of sarcoma.[29] The major difficulty arises in attempting to distinguish carcinoma cells which assume a spindle shape and are arranged in parallel rows, from neoplastic fibroblasts similarly arranged. Since there is almost always a connective tissue stroma with carcinoma, the presence of connective tissue fibers among the tumor cells is not a distinguishing feature. In this regard, the presence of an essentially ectodermal, glial stroma in the brain greatly simplifies this problem. This stroma is composed mainly of astrocytes and their processes, with the oligodendroglia and the microglia, the latter of mesenchymal origin, contributing to only a slight degree. These glial fibrillary processes may be differentiated from collagenous fibers by the staining methods which have been described. In consequence, it is often possible to distinguish a tissue composed of elongated or spindle-shaped glial cells from one composed of fibro-

blasts by the staining characteristics of the accompanying fibers.

Within the substance of the brain, a primary fibrosarcoma is most likely to arise from the blood vessels. The mesenchymal tissues of the brain include the blood vessels, the microglia, and the meninges. The microglial cells have the capacity to form phagocytes but it is to be doubted that they can influence the deposition of connective tissue fibers, since these are not observed in circumstances in which a marked microglial proliferation is noted, as in cerebral infarction. Neoplasms considered to be of such origin are certainly rare, and their delineation from other types of tumors has been a source of confusion.[32] There is little question that the mesenchymal cells of the vessel walls and of the leptomeninges may influence the deposition of connective tissue fibers. Neoplasms of leptomeningeal origin form the common meningioma group, while the occurrence of neoplastic change in the blood vessel walls is reflected in the relative frequency with which primary sarcomas of the brain are characterized as "perithelial."[33] The observations which are considered to implicate the blood vessels as the source of the sarcomatous elements of the tumors being reported have been described (Figs. 2 and 3).

The capacity of the cerebral blood vessels to reactive hyperplasia, particularly in relation to neoplasms, deserves emphasis. In frequency and degree this far exceeds that noted in other organs. In the brain vascular hyperplasia occurs in a variety of circumstances, including anoxia, nutritional deficiencies, and inflammation, but this is comparatively moderate. A very marked vascular hyperplasia occurs almost uniformly in glioblastomas, so that this is often included as a diagnostic criterion for the recognition of this tumor.[4] A similar change may be noted with some other brain tumors as well, including some metastatic tumors.

The relatively slight vascular hyperplasia noted with astrocytoma and the almost constant marked vascular hyperplasia with glioblastoma are of special interest because of recent studies defining a relationship between these tumors. Although considerable controversy remains, we are convinced of the accuracy of the fundamental concept offered by the Mayo Clinic group,[34] in which these tumors and some others are considered to form a single group of varying anaplasia and malignancy. Astrocytoma and glioblastoma bear the same relation to each other as adenoma and adenocarcinoma, except that intermediate forms are much more common, and the transition of the benign to the malignant form more frequently observed. In this sense, the vascular hyperplasia may be described as tending to parallel the de-

gree of malignancy within the group. Studies of these features are contemplated.

An incidental observation relates to case 3 of this report in which the glioblastomatous tissue formed only a minute fraction of the whole tumor, the bulk of which was sarcomatous. There are many reports among those dealing with mixed tumors in other organs which suggest that the sarcomatous elements possess a greater growth potential than the carcinomatous.[26,28-30] The same may be true of the mixed tumors of the brain. The possibility exists that some of the primary sarcomas of the brain, especially some of those characterized as "perithelial," may have arisen by sarcomatous change in the vessels of a glioblastoma with subsequent overgrowth of the sarcoma obscuring or destroying the glioblastoma.

Summary

Three cases of primary brain tumor are described in which the neoplasm was composed of an admixture of dissimilar tissues, both malignant. One component resembled the glioblastoma multiforme; the other, a spindle cell fibrosarcoma.

Evidence is presented to suggest that the sarcomatous elements arose by neoplastic change in the markedly hyperplastic blood vessels regularly seen in the glioblastoma multiforme.

It is suggested that the sarcomatous elements may grow more rapidly than the glioblastomatous tissue, obscuring or obliterating the latter. Some of the primary sarcomas of the brain may have arisen in this fashion.

REFERENCES

1. Lillie, R. D. Histopathologic Technic. Blakiston Co., Philadelphia, 1948, p. 62.
2. Lillie, R. D. Histopathologic Technic. Blakiston Co., Philadelphia, 1948, pp. 195–196.
3. Wilder, H. C. An improved technique for silver impregnation of reticulum fibers. *Am. J. Path.*, 1935, 11, 817–819.
4. Deery, E. M. Some features of glioblastoma multiforme. *Bull. Neurol. Inst. New York*, 1932, 2, 157–193.
5. Stroebe, H. Ueber Entstehung und Bau der Gehirngliome. *Beitr. z. path. Anat. u. z. allg. Path.*, 1895, 18, 405–486.
6. Hildebrandt, K. Zur Kenntnis der gliomatösen Neubildungen des Gehirns mit besonderer Berücksichtigung der ependymären Gliome. *Virchows Arch. f. path. Anat.*, 1906, 185, 341–359.
7. Ewing, J. Neoplastic Diseases: A Treatise on Tumors. W. B. Saunders Co., Philadelphia, 1922, ed. 2, p. 399.
8. Schmincke, A. Ein glioblastisches Sarkom des Kleinhirns mit Metastasenbildung in Hirn und Rückenmark. *Frankfurt. Ztschr. f. Path.*, 1914–15, 16, 357–375.

9. Borst, M. Die Lehre von den Geschwülsten—mit einem mikroscopischen Atlas. J. F. Bergmann, Wiesbaden, 1902, 1, 506–509.

10. Wohlwill, F. Ueber gleichzeitiges Vorkommen von Hirngliom und Sarkom. *Jahrb. d. Hamb. Staatskrankenanst., Hamb. u. Leipz.*, 1911, 15, Pt. 2, 19–32.

11. Globus, J. H., and Strauss, I. Spongioblastoma multiforme. A primary malignant form of brain neoplasm: its clinical and anatomic features. *Arch. Neurol. & Psychiat.*, 1925, 14, 139–191.

12. Bailey, P., and Cushing, H. A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis. J. B. Lippincott Co., Philadelphia, 1926, pp. 73–81.

13. Babes, V. Beobachtungen über Riesenzellen. Bibliotheca Medica, Abteilung C. Erwin Nägele, Stuttgart, 1905, 20, 1–127.

14. Merzbacher and Uyeda. Gliastudien. Das reaktive Gliom und die reaktive Gliose. Ein kritischer Beitrag zur Lehre vom "Gliosarkom." *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1910, 1, 285–317.

15. Bailey, P., and Ley, A. Estudio anatomo-clínico de un caso de ocurrencia simultánea de dos tumores (glioma y sarcoma) en el hemisferio cerebral de un niño. *Arch. de neurobiol.*, 1934, 14, 673–690.

16. Bailey, P.; Buchanan, D. N., and Bucy, P. C. Intracranial Tumors of Infancy and Childhood. University of Chicago Press, Chicago, 1939, pp. 341–345.

17. French, J. D. Astroblastoma and perithelial sarcoma in a case of neoplastic disease of the brain. *J. Neuropath. & Exper. Neurol.*, 1949, 8, 232–239.

18. Nichols, P., Jr., and Wagner, J. A. Primary intracranial sarcoma. Report of nine cases with suggested classification. *J. Neuropath. & Exper. Neurol.*, 1952, 11, 215–234.

19. Davidoff, L. M., and Feiring, E. H. "Circumscribed" glioblastoma multiforme. *J. Neuropath. & Clin. Neurol.*, 1951, 1, 161–167.

20. Watson, T. A. Incidence of multiple cancer. *Cancer*, 1953, 6, 365–371.

21. Bugher, J. C. The probability of the chance occurrence of multiple malignant neoplasms. *Am. J. Cancer*, 1934, 21, 809–824.

22. Slaughter, D. P. The multiplicity of origin of malignant tumors: collective review. *Surg., Gynec. & Obst.*, 1944, 79, 89–98.

23. Warren, S., and Ehrenreich, T. Multiple primary malignant tumors and susceptibility to cancer. *Cancer Research*, 1944, 4, 554–570.

24. Saphir, O., and Vass, A. Carcinosarcoma. *Am. J. Cancer*, 1938, 33, 331–361.

25. Willis, R. A. Pathology of Tumours. C. V. Mosby Co., St. Louis, 1953, ed. 2, pp. 139–141.

26. Harvey, W. F., and Hamilton, T. D. Carcino-sarcoma. A study of the microscopic anatomy and meaning of a peculiar cancer. *Edinburgh M. J.*, 1935, 42, 337–378.

27. Bonser, G. M., and Orr, J. W. The morphology of 160 tumours induced by carcinogenic hydrocarbons in the subcutaneous tissues of mice. *J. Path. & Bact.*, 1939, 49, 171–183.

28. Ehrlich, P., and Apolant, H. Beobachtungen über maligne Mäusetumoren. *Berl. klin. Wchnschr.*, 1905, 42, 871–874.

29. Ludford, R. J., and Barlow, H. Sarcomatous transformation of the stroma of mammary carcinomas that stimulated fibroblastic growth *in vitro*. *Cancer Research*, 1945, 5, 257–264.

30. Taylor, A., and Carmichael, N. Stroma malignancy in mouse-grown transplants of egg-cultivated mouse mammary carcinoma. *Cancer Research*, 1947, 7, 78–87.

31. Goldfeder, A., and Nagasaki, F. Spontaneous transformation from carcinomatous to sarcomatous-like growth. *Cancer Research*, 1954, 14, 267–270.

32. Russell, D. S.; Marshall, A. H. E., and Smith, F. B. Microgliomatosis. A form of reticulosis affecting the brain. *Brain*, 1948, 71, 1–15.

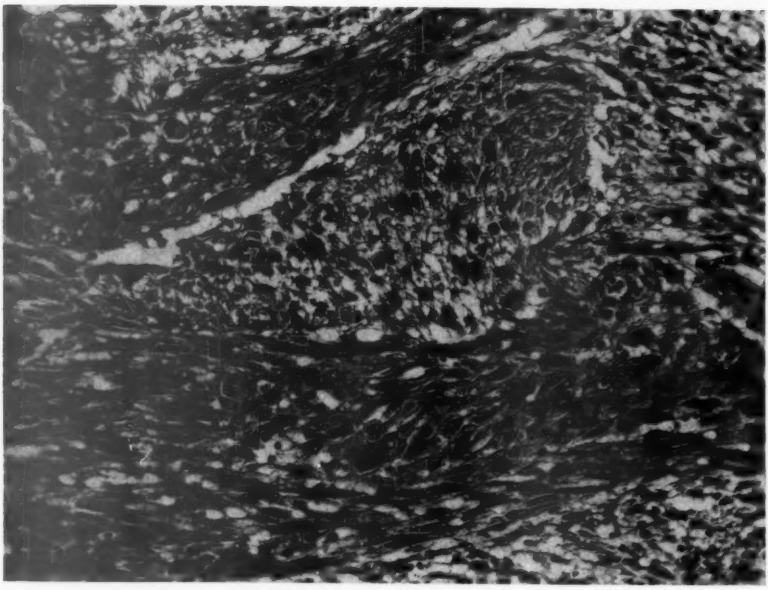
33. Hanbery, J. W., and Dugger, G. S. Perithelial sarcoma of the brain—a clinicopathological study of thirteen cases. *A. M. A. Arch. Neurol. & Psychiat.*, 1954, 71, 732–761.

34. Svien, H. J.; Mabon, R. F.; Kernohan, J. W., and Adson, A. W. Astrocytomas. *Proc. Staff Meet., Mayo Clin.*, 1949, 24, 54–64.

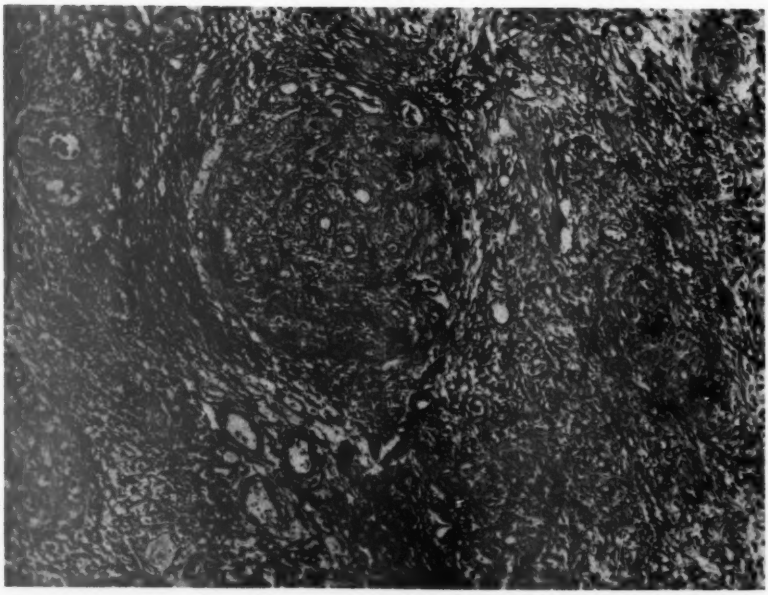
LEGENDS FOR FIGURES

FIG. 1. Case 1. Tumor, showing admixture of glioblastomatous and sarcomatous tissues. Hematoxylin and eosin stain. × 150.

FIG. 2. Case 1. Tumor, showing circumscribed islands of sarcomatous tissue encompassed by glioblastomatous tissues. Azocarmine stain. × 80.



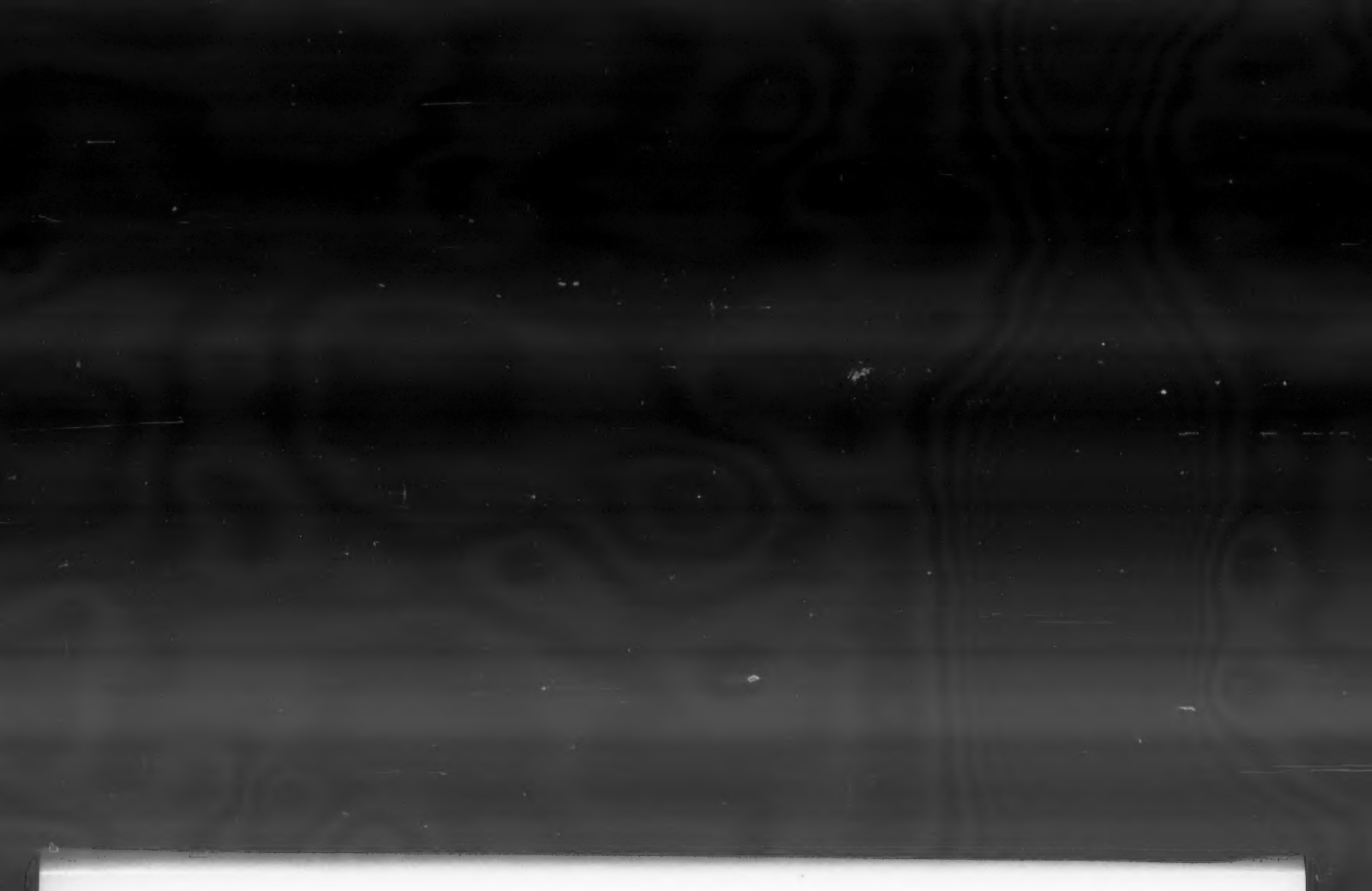
1

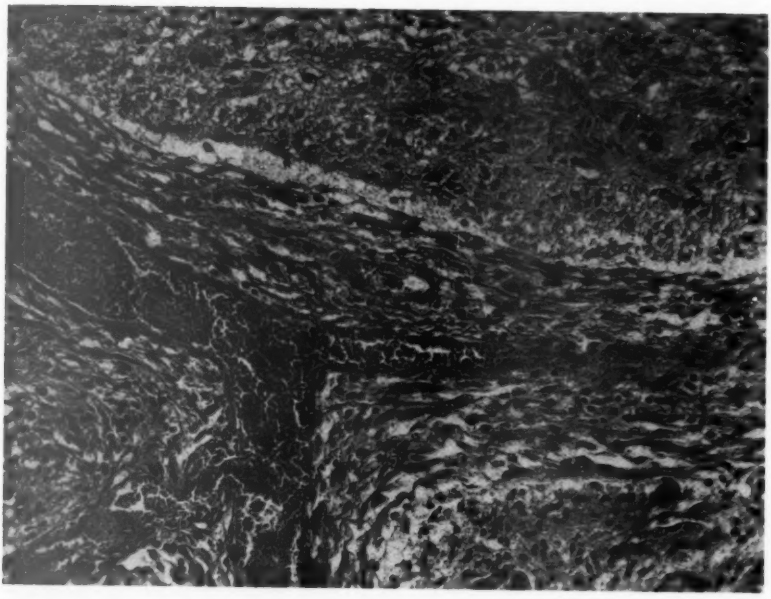


2

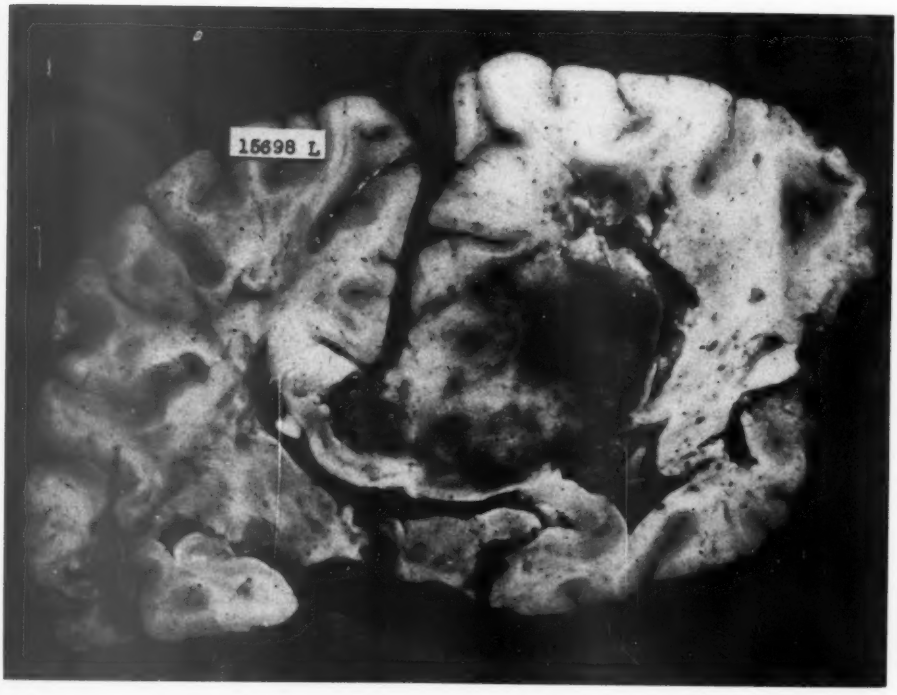
FIG. 3. Case 2. Tumor, showing variability and disorganization of the cells of a blood vessel within a glioblastomatous area. Hematoxylin and eosin stain. × 170.

FIG. 4. Case 2. Coronal section of brain showing position and gross appearance of tumor. × 7/8ths.





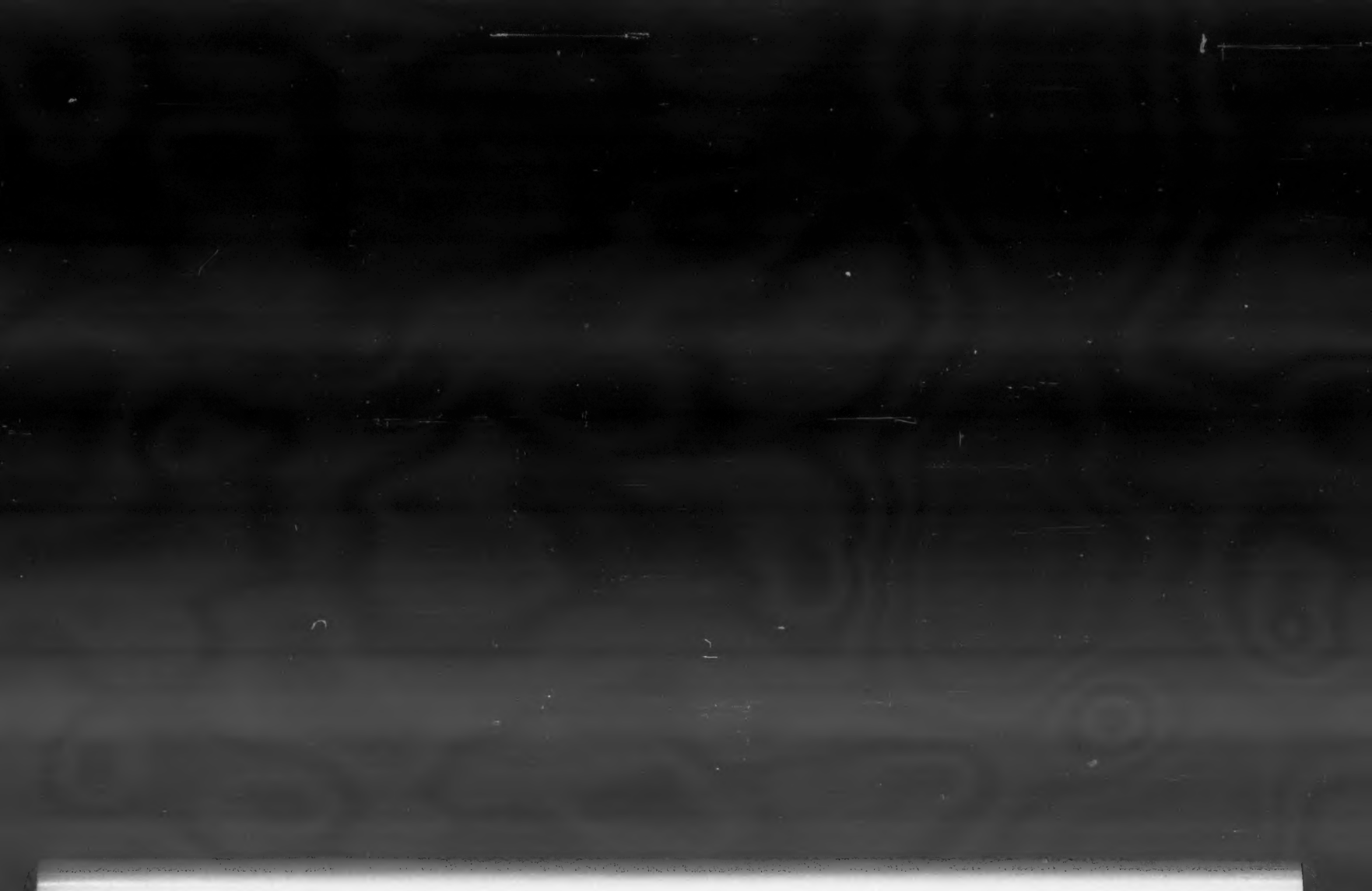
3

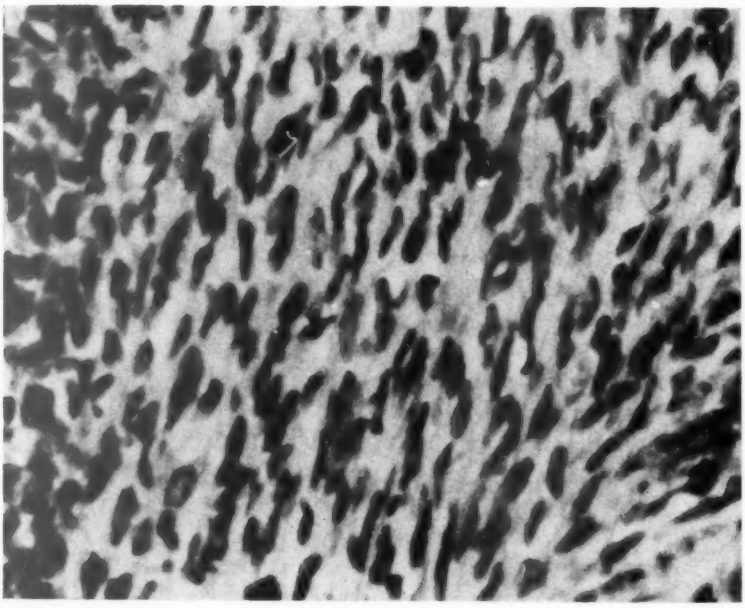


4

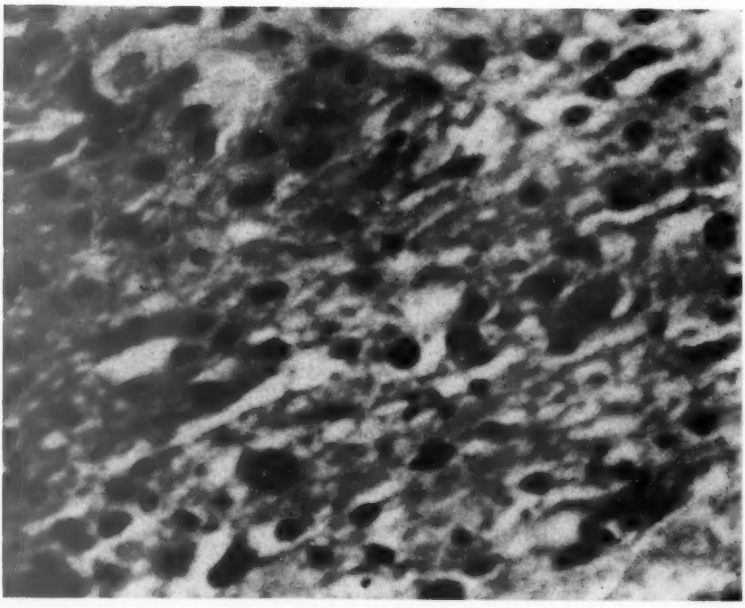
FIG. 5. Case 3. Tumor removed at surgery, showing sarcomatous features. Hematoxylin and eosin stain. × 550.

FIG. 6. Case 3. Portion of tumor in necropsy specimen, showing glioblastomatous area. Hematoxylin and eosin stain. × 530.





5



6

MORPHOLOGIC CHANGES IN RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MENINGOCOCCAL TOXIN

II. Two Appropriately Spaced Injections; the Rôle of Fibrinoid in the Generalized Shwartzman Reaction *

Joel G. Brunson, M.D.†; Lewis Thomas, M.D.,‡ and Charles N. Gamble, M.D.
From the Department of Pathology (Drs. Brunson and Gamble) and the Pediatric Research Laboratories of the Heart Hospital (Dr. Thomas), University of Minnesota Medical School, Minneapolis 14, Minn.

The morphologic changes which occur in rabbits following a single intravenous injection of meningococcal endotoxin have been described in the preceding paper.[1] Reference was made to the fact that, under certain circumstances, one injection may be followed by bilateral renal cortical necrosis. With two appropriately spaced intravenous injections of endotoxin, this reaction is regularly produced and is commonly referred to as the generalized Shwartzman phenomenon. The renal lesion, as shown by Apitz,[2,3] Gratia and Linz,[4] Gerber,[5] Black-Schaffer, Hiebert, and Kerby,[6] and others is the characteristic gross lesion of the generalized phenomenon. Previous studies of this lesion[7] have shown that it is initiated by occlusion of the glomerular capillaries by an eosinophilic, homogeneous material resembling fibrinoid, which appears within a few hours after the second injection of toxin. The hemorrhage and necrosis of the renal cortex is considered to be the result of interference with glomerular circulation.

Changes have been described also in other organs. Apitz,[3] Shwartzman *et al.*,[8] and Gerber[5] observed occasional areas of necrosis in the myocardium, and venous thrombi and necrosis in the liver. Black-Schaffer *et al.*[6] noted these lesions and also described a type of pulmonary arteritis and hyperplasia of the reticulo-endothelial cells in the spleen. The occurrence of bilateral adrenal hemorrhage and necrosis was noted also in a small number of animals. Thomas, Denny, and Floyd[9] recently reported that an intravenous injection of meningococcal toxin in rabbits previously infected with group A hemolytic streptococci was followed in approximately 50 per cent of the animals by the development of cardiac lesions characterized by the presence

* This study was aided by grants from the Minnesota Heart Association, the American Heart Association, and the Youngstown Area Heart Association. The first paper of this series appeared in the May–June issue.

Received for publication, November 2, 1954.

† Fellow of the American Cancer Society.

‡ Now at Department of Pathology, New York University—Bellevue Medical Center, New York, N.Y.

of fibrinoid beneath the endothelium of the coronary arteries and within the valves. A majority of the animals also developed bilateral renal cortical necrosis identical with that occurring in the generalized Shwartzman phenomenon. The material in the glomerular capillaries was morphologically and tinctorially similar to that noted in the coronary arteries and heart valves. Similar renal and cardiac lesions were described in rabbits infected with pneumococci and given an intravenous injection of meningococcal toxin.[10] Such cardiac lesions were not observed in control animals given meningococcal toxin, and it was suggested in these reports that bacterial infection might play a primary rôle in the localization of fibrinoid in the heart.

As noted in the preceding paper,[1] the majority of mature animals given a single intravenous injection of meningococcal toxin were found to develop cardiac fibrinoid, and similar material occasionally appeared in the hearts of immature rabbits. On the basis of these observations a more detailed study of the changes following two intravenous injections of endotoxin was undertaken, and this paper deals with the changes produced by two such injections, the chronologic development of the various lesions, and their association with the presence of fibrinoid.

Material and Methods

Hybrid albino rabbits of mixed sexes, weighing 1 to 1.5 kg., were used. They were fed Purina rabbit pellets and had free access to water.

Meningococcal toxin was used throughout the experiments. Details of its preparation have been reported elsewhere.[7] It was diluted 1:80 with sterile, pyrogen-free, saline solution and injected into the marginal ear vein of the animal in a volume of 2 cc. Two intravenous injections of this material, with intervals of 8 to 72 hours between the injections, were given to 38 animals.

Following death or sacrifice of the animals, routine post-mortem examinations were performed and the tissues were fixed in 10 per cent neutral formalin. Sections from some of the organs were fixed in a solution containing equal volumes of 8 per cent basic lead acetate solution and 15 per cent formalin, for special stains. Sections from the lungs, heart, liver, pancreas, spleen, adrenal glands, skeletal muscle, and kidneys were studied routinely and sections from other organs occasionally. The heart was sectioned so as to reveal the valves and the interventricular septum.

Hematoxylin and eosin were used routinely and certain additional

sections were stained by the periodic acid-Schiff method. Selected sections from the kidneys and spleens were stained by the toluidine blue method of Lison,[11] Mallory's phosphotungstic acid hematoxylin, crystal violet, and van Gieson's stain.

Results

Of the total number of animals, 14 (37 per cent) died and the remainder were killed at intervals of 24 to 96 hours following the second injection. The structural changes involving the heart, kidneys, spleen, liver, and lungs are summarized in Table I. From this table it may be seen that while certain lesions occurred regardless of the interval between the two injections, the incidence of such changes was generally higher when the interval was 21 or 24 hours.

Kidney. Slightly more than 50 per cent of the animals developed bilateral renal cortical necrosis. In the fully developed stage of the lesion (24 hours following the second injection of endotoxin) the kidneys were enlarged grossly, had a tense capsule, and a bluish, cyanotic color. The surface showed many petechial hemorrhages and areas of

Table I

Changes Following Two Intravenous Injections of Meningococcal Toxin (Dilution 1:80) in Small Rabbits

Interval between injections	Number of rabbits	Number with renal lesions	Number with splenic fibrinoid and necrosis	Number with hepatic thrombi and necrosis	Number with pulmonary thrombi	Number with cardiac lesions	Number with cardiac fibrinoid
hrs.							
8	7	2	3	3	1	6	0
21	4	3	4	3	0	2	3
24	7	5	5	5	2	6	4
56	16	9	9	11	7	15	9
72	4	1	2	2	1	4	0

grayish white necrosis. The cut surface showed irregular areas of extensive hemorrhage and necrosis which often extended into the medulla (Fig. 1).

At earlier stages the changes were less pronounced and the kidneys usually were deep red with superimposed petechial hemorrhages. On cut section the cortex showed many discrete, circumscribed, grayish white areas of necrosis, and the medulla appeared deeply congested (Fig. 2). Occasionally, distinct triangular areas of infarction were noted, although no thrombi were demonstrated in the larger renal vessels.

Microscopic examination disclosed a variable pattern, depending on the age of the lesion. In late stages diffuse hemorrhage and necrosis, involving most of the cortex and medulla, were noted, and almost every glomerulus appeared occluded by dense, refractile, homogeneous, eosinophilic material. Similar material was noted in the lumen of arterioles and occasionally in the lumen and beneath the endothelium of arteries (Fig. 5). Often the tubules were not recognizable as such and appeared as solid casts of smooth, eosinophilic material.

Earlier stages showed less pronounced alterations consisting of scattered areas of hemorrhage and tubular necrosis, with variable numbers of occluded capillary loops. The lumina of many of the tubules contained deeply eosinophilic, homogeneous casts, similar to the material in the glomerular capillaries. By study of serial sections, it was possible in some cases to trace a communication between these casts and the material in the capillary loops.

Sections of the kidneys containing this material were subjected to special staining procedures, with the following results: With Mallory's phosphotungstic acid-hematoxylin, a homogeneous purple to orange-purple with occasional purple fibrils (Fig. 6); with van Gieson's stain, yellow; with crystal violet, purple to reddish purple; with toluidine blue, homogeneous blue; and with the periodic acid-Schiff stain, deep reddish purple (Fig. 5). Casts present in the tubules stained in a similar manner.

The material was examined also in the fresh state. The cortical portion of kidneys showing gross necrosis was dissected, mixed with isotonic saline solution, and placed in a Waring blendor for 3 minutes. The material was then allowed to settle by gravity and the resulting supernatant centrifuged at 500 r.p.m. for 3 minutes. Portions of the sediment from this process were placed on a microscopic slide and studied by means of polarized light.

Many intact glomeruli were observed; these appeared larger than those from normal rabbit kidneys. A high degree of birefringence (Figs. 3 and 4) was demonstrated in a majority of the glomeruli, which was not present in normal kidney preparations. In some instances only portions of the glomerulus were birefringent, indicating that certain loops did not contain the material. The degree of birefringence also varied with the age of the lesion, being greater in those in which the reaction had reached a maximum. Glomeruli extracted in this manner and placed in the refrigerator for as long as 10 days still retained a high degree of birefringence. The birefringence was unaffected by extraction with a solution containing equal parts of absolute

alcohol and acetone, or by alcohol alone, but disappeared within a period of 10 to 15 minutes when the material was exposed to 1 per cent crystalline papain or 1 per cent trypsin. Birefringence was not impaired after exposure to pH 10 buffer solutions for 18 hours, but disappeared rapidly in a solution of pH 2.5. These observations suggest that the intracapillary material causing birefringence is a highly organized protein, susceptible to proteolysis and acid hydrolysis.

Spleen. Alterations in the spleen were found in 61 per cent of the total group, and these changes, like the renal lesion, were increased in frequency when the interval between the two injections of toxin was 21 or 24 hours. Grossly, 24 hours after the second injection of toxin, the organ was distinctly enlarged, its capsule appeared stretched, and it was dark purple-red. It also felt much firmer than normal and occasionally was "board-like" on palpation. Over the surface of the organ distinct areas of grayish white necrosis frequently were observed. On the cut surface these showed a surrounding hemorrhagic zone and resembled typical areas of infarction.

Microscopically, the changes were strikingly similar to those observed in the spleens from mature rabbits given a single injection of endotoxin,[1] i.e., the sinusoids contained a homogeneous, deeply eosinophilic, refractile material similar to that described in the glomerular capillaries (Fig. 7). Chronologic study of the development of the splenic lesion showed that as early as 1 hour after the second injection of toxin the sinusoids contained clumps of this material, which at this time appeared slightly vacuolated and contained nuclear débris. The sinusoids also appeared markedly congested at this time with great numbers of conglutinated erythrocytes.

During a period of 8 to 12 hours following the second injection of endotoxin this eosinophilic material continued to accumulate, with gradual obliteration of the sinusoids and hemorrhagic necrosis of portions of the organ. Sections from the spleen occasionally appeared as hemorrhagic masses of tissue with remaining islands of lymphatic corpuscles. In some animals typical infarcts were observed, but no thrombi were noted in the larger splenic vessels. Sections of the spleens containing this material were subjected to the same special stains as the kidney sections, and identical results were obtained.

Liver. Gross examination of the liver usually failed to reveal any striking changes, although occasionally it was possible to discern petechial hemorrhages and punctate, gray areas resembling areas of necrosis. Microscopically, areas of focal necrosis and venous thrombi were noted in 63 per cent of the animals. The necrotic foci sometimes

were hemorrhagic and sometimes pale, and often showed a surrounding cellular reaction of mononuclear and heterophilic cells. The thrombi lay in the lumina of efferent veins and consisted of irregular clumps of material with large numbers of enmeshed red and white blood cells and nuclear débris. In several instances thrombi were covered by endothelium and projected into the lumen of the vessel. Some of the thrombi were Schiff-positive; others were not.

Lungs. Grossly, the lungs displayed hemorrhages and, at times, hemorrhagic infarcts. Microscopically, 28 per cent of the cases showed thrombi or emboli within large and small pulmonary arteries. These were similar morphologically and tinctorially to those in the liver.

Heart. Gross changes in the heart were similar to those described as occurring in animals given one intravenous injection of endotoxin. They consisted of areas of hemorrhage and grayish white discolorations of the muscle.

Microscopically, 87 per cent of the hearts showed areas of hemorrhage, muscle necrosis and cellular reaction, calcification of muscle fibers, and valvular hemorrhages. In 3 cases, granular thrombotic material was noted in the intramural coronary arteries. These changes in the heart were similar to those described previously,[1] but were much more extensive. The coronary arteries displayed marked changes, with fragmentation of the endothelium and large vacuoles in the intima and media. Many of the vessels showed a pronounced reaction of mononuclear cells and cells with oval nuclei and a central longitudinal bar of chromatin, resembling Anitschkow cells. At times these appeared to be originating directly from the muscle of the arterial media, and cells in mitosis were observed occasionally in the muscle of the media.

In 42 per cent of the total group, fibrinoid was found in the coronary arteries and within the heart valves. The lesions were strikingly similar to those previously described in rabbits given endotoxin after the establishment of systemic streptococcal infection.[9] Morphologically, it appeared similar to the material seen in the glomerular capillaries and in the splenic sinusoids. It was deep reddish purple when stained by the periodic acid-Schiff method (Fig. 8). In the arteries it often appeared as a layer of homogeneous material lying just beneath the endothelium, but occasionally appeared in the form of droplets which extended outward into the media. In other vessels it appeared to be largely subendothelial and to push into the lumen. Serial sections of some of these blocks showed a communication between this material and similar material lying free in the lumen.

The valvular changes were of particular interest. Hemorrhage was

noted frequently in association with the presence of fibrinoid, and at times overshadowed the latter to a considerable extent. In some valves there was a significant degree of cellular reaction about the fibrinoid and many of the cells exhibited the characteristics of Anitschkow cells. This cellular reaction was encountered occasionally in valves in which no fibrinoid was observed (Figs. 9 and 10).

Other Changes. In one animal an area of hemorrhage approximately 1 cm. in length was observed in the small intestine. Microscopically, in addition to hemorrhage, there were vascular changes consisting of the presence of fibrinoid in the media of the arteries and a surrounding cellular reaction of mononuclear and heterophilic cells.

Sections from the adrenal glands, pancreas, and skeletal muscle showed no gross or microscopic changes.

Discussion

The changes which have been described in the kidneys appear to result from gradual occlusion of the glomerular capillaries. The morphologic features of the occluding material within the capillaries suggest that it is fibrinoid. The tinctorial features strongly support this view, since it has all of the properties which Altshuler and Angevine[12] attributed to this material. The birefringent properties and reaction of the material to proteolytic enzymes suggest that it is a highly organized protein compound.

Morphologically, it appears to be carried to the kidney by the blood rather than to develop within the organ. The presence of similar material within the lumen of the afferent arteriole and the rapid appearance of the material following a second injection of endotoxin support this view. Kane, Good, and Thomas[13] made a detailed study of the development of the renal lesion and found that Schiff-positive material lining the capillary basement membranes could be detected within a period of 2 hours after the second injection of endotoxin.

Similarly, in the development of the splenic lesion, the earliest change is the presence of this material within the sinusoids. In this organ, as in the kidney, it appears to accumulate over a period of hours and gradually to obstruct the blood flow. Further, the character of the lesions noted in the lungs, heart, and liver suggests an occlusive vascular phenomenon involving predominantly the capillaries and arterioles with resultant areas of ischemic necrosis.

It has been suggested that spasm of the renal vessels plays an important part in the genesis of cortical necrosis.[14] It would appear, however, that if this factor plays any definite rôle, it is secondary to

vascular occlusion. In these studies, at any rate, necrosis of the renal tubules was never observed in the absence of capillary occlusion, and the latter is the earliest morphologic change to be observed in the development of the renal lesion. Other experimental observations[15] suggest that renal vascular spasm, at least at the time of the second injection of endotoxin, inhibits the development of renal cortical necrosis. Moreover, it does not seem reasonable to assume that spasm plays an important part in the development of areas of necrosis in the spleen.

The incidence of fibrinoid in the hearts in this series of animals does not differ appreciably from that reported as occurring in animals subjected to streptococcal or pneumococcal infection and then given endotoxin.[9,10] Further evidence that this material is not specifically related to bacterial infection was obtained by a review of material from a series of animals given an injection of colloidal iron followed by an injection of meningococcal toxin. The administration of colloidal iron to small rabbits followed at short intervals by an injection of meningococcal toxin produces bilateral renal cortical necrosis in a majority of them.[16] In a series of 18 animals in which this procedure had been carried out, renal cortical necrosis occurred in approximately 70 per cent, and fibrinoid was found in the coronary arteries and heart valves in approximately 30 per cent. With hematoxylin and eosin stains, it was noted that some of the fibrinoid in the coronary arteries was mixed with a brown pigment, and similar pigment was mixed with the material in the glomerular capillaries. This pigment gave a positive reaction for iron when stained by Perls' method. The presence of iron in association with fibrinoid supports the hypothesis that the latter material is derived from a component of the circulating blood.

On the basis of the morphologic changes which have been described, and their chronologic development, it would appear reasonable to conclude that some alteration in the circulating blood accounts for the formation of this material which has the morphologic and tinctorial features of fibrinoid.

Summary and Conclusions

Thirty-eight albino rabbits of mixed sexes, weighing 1 to 1.5 kg., were given two intravenous injections of meningococcal toxin at intervals of 8 to 72 hours. Of these, 37 per cent died and the remainder were killed at various intervals following the second injection.

The important feature of the pathologic changes following such a

procedure is the presence of occlusive masses of fibrinoid in the glomerular capillaries, the splenic sinusoids, beneath the endothelium of the coronary arteries, and within the heart valves. The accumulation of this material in the kidneys and spleen results in the development of renal cortical necrosis and splenic hemorrhages and necrosis. The renal and splenic lesions occurred in 53 and 61 per cent of the animals, respectively, and fibrinoid was found in the hearts in 42 per cent. Thrombi and necrosis in the liver were observed in 63 per cent of the animals, and similar thrombi or emboli were noted in branches of the pulmonary arteries in 28 per cent.

The character of the lesions, their widespread distribution, and their chronologic development suggest an occlusive vascular phenomenon involving predominantly the capillaries and small arteries with resulting ischemic necrosis in various organs. The occlusive material has the morphologic and tinctorial features of fibrinoid, and these features of the lesions suggest that fibrinoid may be derived from the circulating blood.

The photographs were made by Mr. Henry Morris.

REFERENCES

1. Brunson, J. G.; Gamble, C. N., and Thomas, L. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. I. The effects produced in young and in mature animals by a single injection. *Am. J. Path.*, 1955, 31, 489–499.

2. Apitz, K. Die Wirkung bakterieller Kulturfiltrate nach Umstimmung des gesamten Endothels beim Kaninchen. *Virchows Arch. f. path. Anat.*, 1934, 293, 1–33.

3. Apitz, K. A study of the generalized Shwartzman phenomenon. *J. Immunol.*, 1935, 29, 255–266.

4. Gratia, A., and Linz, R. Les phénomènes de Sanarelli et de Shwartzman ou l'allegie hémorragique. *Ann. Inst. Pasteur*, 1932, 49, 131–185.

5. Gerber, I. E. The Shwartzman phenomenon in the kidneys of rabbits. Observations on effects of intravenous administration of bacterial filtrates. *Arch. Path.*, 1936, 21, 776–796.

6. Black-Schaffer, B.; Hiebert, T. G., and Kerby, G. P. Experimental study of purpuric meningococcemia in relation to the Shwartzman phenomenon. *Arch. Path.*, 1947, 43, 28–54.

7. Thomas, L., and Good, R. A. Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. *J. Exper. Med.*, 1952, 96, 605–624.

8. Shwartzman, G.; Klemperer, P., and Gerber, I. E. The phenomenon of local tissue reactivity to bacterial filtrates. The rôle of altered vascular response in certain human diseases. *J. A. M. A.*, 1936, 107, 1946–1951.

9. Thomas, L.; Denny, F. W., Jr., and Floyd, J. Studies on the generalized Shwartzman reaction. III. Lesions of the myocardium and coronary arteries accompanying the reaction in rabbits prepared by infection with group A streptococci. *J. Exper. Med.*, 1953, **97**, 751–766.

10. Good, R. A.; Thomas, L., and Smith, R. T. Unpublished observations.

11. Glick, D. Techniques of Histo- and Cytochemistry. Interscience Publishers, Inc., New York, 1949, pp. 46–47.

12. Altshuler, C. H., and Angevine, D. M. Histochemical studies on the pathogenesis of fibrinoid. *Am. J. Path.*, 1949, **25**, 1061–1077.

13. Kane, D. J.; Good, R. A., and Thomas, L. Histologic studies of the renal lesion in the generalized Shwartzman phenomenon. (Abstract.) *Anat. Rec.*, 1952, **112**, 349.

14. Sheehan, H. L., and Moore, H. C. Renal Cortical Necrosis and the Kidney of Concealed Accidental Haemorrhage. Charles C Thomas, Springfield, Ill., 1953, 186 pp.

15. Brunson, J. G., and Gamble, C. N. Unpublished observations.

16. Smith, R. T.; Thomas, L., and Good, R. A. Generalized Shwartzman reaction. V. Intravenous injection of colloidal iron or carbon on response of rabbits to meningococcal toxin. *Proc. Soc. Exper. Biol. & Med.*, 1953, **82**, 712–715.

LEGENDS FOR FIGURES

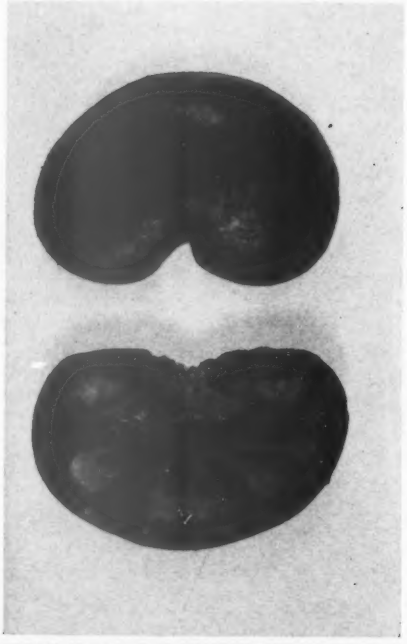
FIG. 1. Gross appearance of the kidney from a rabbit killed 36 hours after a second intravenous injection of meningococcal toxin. The capsular surface shows marked hemorrhage and necrosis and the cut surface shows extensive necrosis involving both cortex and medulla. Photographed after fixation in 10 per cent formalin.

FIG. 2. Kidney from a rabbit killed 12 hours after a second intravenous injection of meningococcal toxin. There are discrete areas of necrosis in the cortex and marked medullary congestion. Formalin fixation.

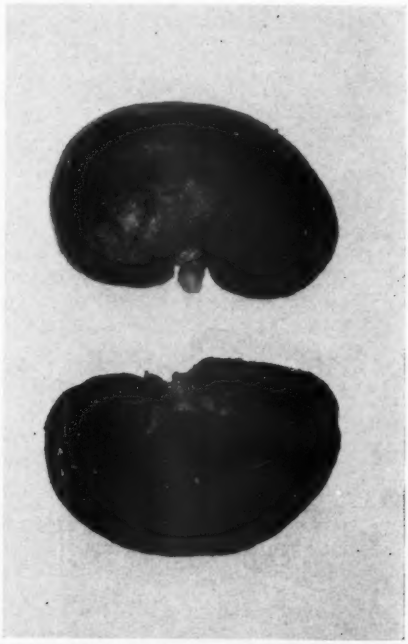
FIG. 3. Isolated glomerulus from the kidney of a rabbit killed 18 hours after a second intravenous injection of meningococcal toxin. Photographed under normal light. × 100.

FIG. 4. The same glomerulus seen in Figure 3, photographed under polarized light. Birefringent material is present in the capillary loops. × 100.

1



2



3

4

FIG. 5. Section from the kidney of a rabbit stained by the periodic acid-Schiff method. Counterstained with celestin blue. Schiff-positive material lines the glomerular capillary basement membranes and similar material is present within the lumen and in the wall of the afferent arteriole. The animal died approximately 80 hours after a second intravenous injection of meningococcal toxin. × 450.

FIG. 6. Section from the kidney of a rabbit which died 48 hours after a second intravenous injection of meningococcal toxin. Mallory's phosphotungstic acid-hematoxylin stain. The material within the glomerular capillary loops is purplish red and there are casts within the lumina of the tubules. × 450.

FIG. 7. Section from a spleen showing the accumulation of homogeneous, eosinophilic material in the splenic sinusoids. Rabbit killed 24 hours after a second intravenous injection of meningococcal toxin. Hematoxylin and eosin stain. × 100.

FIG. 8. Intramural coronary artery showing the presence of fibrinoid beneath the endothelium. From a rabbit killed 24 hours after a second intravenous injection of meningococcal toxin. Periodic acid-Schiff's stain. × 450.

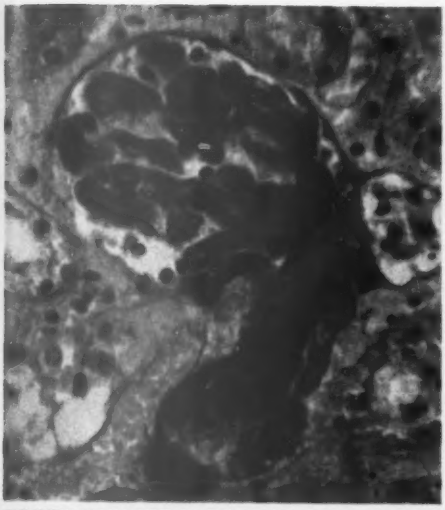
FIG. 9. Section from a cardiac valve of an animal which died 88 hours after a second intravenous injection of meningococcal toxin. An accumulation of fibrinoid within the substance of the valve leaflet may be noted. Hematoxylin and eosin stain. × 200.

FIG. 10. Cardiac valve from another rabbit which died 88 hours after a second injection of meningococcal toxin, showing fibrinoid together with many "owl-eyed" cells within the leaflet. Hematoxylin and eosin stain. × 200.

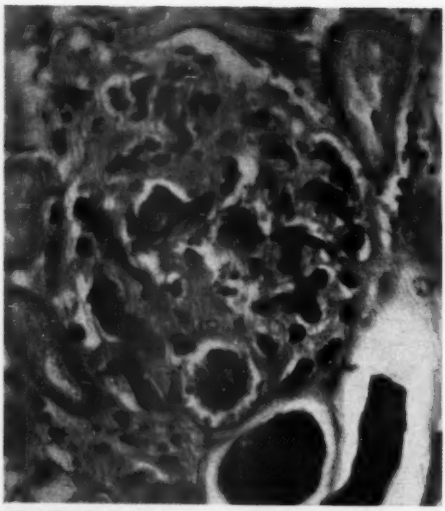
5

7

9



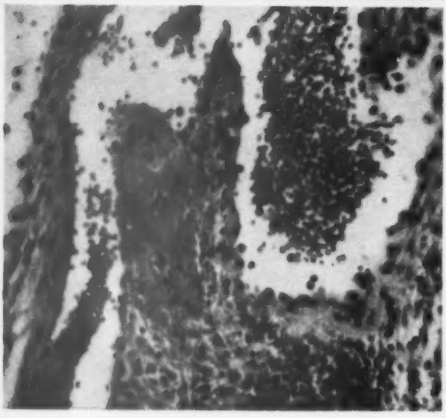
5



6

7

8



9

10

MORPHOLOGIC CHANGES IN RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MENINGOCOCCAL TOXIN

III. The Effects Produced by Endotoxin in Association with Certain High Molecular Weight Acidic Polymers *

Joel G. Brunson, M.D.†; Richard L. Davis, B.A., and Lewis Thomas, M.D.‡
From the Department of Pathology (Dr. Brunson and Mr. Davis) and the Pediatric Research Laboratories of the Heart Hospital (Dr. Thomas), University of Minnesota Medical School, Minneapolis 14, Minn.

Previous papers[1,2] have dealt with the lesions occurring in rabbits following the intravenous administration of meningococcal endotoxin. The present paper reports the results of experiments designed to study the effects produced by meningococcal toxin when given in conjunction with certain high molecular weight acidic polymers, specifically sodium polyanetholsulfonate, the sodium salt of polyvinyl alcohol polysulfonic acid ester (PVAS), and dextran sulfate. Interest in these compounds was stimulated by the reports of Hausman and Dreyfus[3] and Walton[4] concerning the effects produced by these substances in animals and by the studies of Inderbitzin[5,6] concerning the experimental production of lupus erythematosus (LE) cells.

Sodium polyanetholsulfonate is a high molecular weight polymer of sodium anetholsulfonate manufactured by Hoffmann-La Roche, Inc., under the trade name of Liquoid-Roche. It has been used as an adjuvant in blood cultures[7] and as an anticoagulant *in vitro*,[8] but its exact mechanism of action is not known. Evidence indicates that at least part of its activity is due to a combination with fibrinogen.[9] Demole and Reinert[10] reported the development of widespread hemorrhages and a type of "glomerulonephritis" in rabbits given intravenous injections of Liquoid. Hausman and Dreyfus[3] described the occurrence of "intracapillary precipitates" in the renal glomeruli of rabbits given a single intravenous injection of a large amount of Liquoid, and similar precipitates with associated tubular necrosis were observed in rabbits given multiple injections of the material. The capillary lesions were described as being similar to the "wire loops" of disseminated lupus erythematosus and consisted of a homogeneous, acidophilic material lining the capillary basement membranes. The material was

* This study was supported by grants from the Minnesota Heart Association, the American Heart Association, and the Youngstown Area Heart Association.
Received for publication, November 2, 1954.
† Fellow of the American Cancer Society.
‡ Now at Department of Pathology, New York University—Bellevue Medical Center, New York, N.Y.

purple-red when stained by the periodic acid-Schiff method and purple with Weigert's fibrin stain. Similar material was noted in the pulmonary capillaries and in the liver sinusoids. These writers also described the occurrence of petechial hemorrhages in the thymus, heart, and lungs. In all but one animal, bilateral adrenal hemorrhage was observed.

Inderbitzin,[5] in 1952, reported the production of cells resembling LE cells by the addition of Liquoid to suspensions of normal leukocytes. In a later paper,[6] however, he reported that the inclusions in these cells appeared to represent phagocytosed platelets and nuclear débris rather than the amorphous material associated with true LE cells. In addition, he described the production of cells identical with those occurring in lupus erythematosus by the use of the sodium salt of the ester (PVAS) of polyvinyl alcohol and polysulfonic acid.

Little has been written concerning the biologic properties of PVAS. Patat and Vogler,[11] in 1952, described the preparation of a series of such substances and showed that they had anticoagulant properties somewhat similar to heparin. They also noted that the anticoagulant activity and the toxicity increased with increasing degrees of polymerization and sulfur content.

Reports by Ricketts and Walton[12] and Walton[4] concerning the biologic properties of certain dextran sulfate preparations indicated that these substances might have an action similar to that of Liquoid and PVAS. Walton[4] described the occurrence of "fibrin emboli" in the liver, spleen, lungs, and kidneys of animals given intravenous injections of dextran sulfates of varying molecular weights. These "emboli" were metachromatic when stained with toluidine blue, and similar metachromatic material was noted in the Kupffer cells of the liver and the reticulo-endothelial cells of the spleen. He reported that the toxicity of the dextran sulfates varied with the molecular weight; those of higher weights forming insoluble complexes with fibrinogen and producing agglutination of erythrocytes and platelets to form conglutination thrombi.

The renal lesion described by Hausman and Dreyfus[3] and Walton[4] seemed strikingly similar to that produced by two appropriately spaced intravenous injections of endotoxin (the generalized Shwartzman phenomenon). As reported previously,[2] the formation of fibrinoid is a characteristic feature of this phenomenon. It therefore seemed of interest to determine what changes might occur if endotoxin were given in conjunction with sodium polyanetholsulfonate, and the major portion of this report deals with those changes.

The results of these experiments then prompted us to investigate the changes occurring in animals given endotoxin in association with the sodium salt of PVAS, and with dextran sulfate. A more detailed account of these changes is the subject of another report.[13]

Materials and Methods

Meningococcal endotoxin, prepared by a method described previously,[14] was used throughout the experiments. It was diluted in sterile isotonic saline solution and injected in the marginal ear vein in a volume of 2 cc.

Liquoid and PVAS were obtained from Hoffmann-La Roche, Inc. The molecular weight of Liquoid is not known, but that of PVAS is reported to be approximately 40,000 and it has a sulfur content of approximately 18 per cent.[15] Dextran sulfate was prepared by the method of Ricketts and Walton.[12] These materials were dissolved in sterile, pyrogen-free, isotonic saline solution, passed through a Seitz filter, and injected in various doses in the marginal ear vein. Cultures of the filtered material were consistently sterile. In the experiments, 174 hybrid albino rabbits of mixed sexes were used. They were fed Purina rabbit pellets and had free access to water. In view of the previously described differences in reactivity of rabbits of different ages,[1] two sizes of animals were used. The "small" rabbits weighed between 1 and 1.5 kg., and the "large" rabbits weighed between 4 and 4.5 kg. Details concerning the numbers of animals injected with the materials under study are given in the tables.

The animals died or were killed at intervals ranging from 30 minutes to 96 hours following the last injection. Routine post-mortem examinations were performed and the tissues were fixed in 10 per cent neutral formalin. The hearts were sectioned so as to reveal the interventricular septum and the valves, and the brains were cut so as to include the choroid plexus. Hematoxylin and eosin were used routinely and selected sections from various organs were stained by one or more of the following methods: periodic acid-Schiff's, toluidine blue, phosphotungstic acid-hematoxylin, crystal violet, and van Gieson's stain.

Results

Lethal Effect

Liquoid Alone. In a dose of 4 mg., Liquoid caused no deaths, but with increasing amounts a corresponding increase in lethal effect was noted. With 32 mg., a majority of the small animals died within 12 hours following the injection. In the large rabbits, 16 mg. produced

death in one of 5 animals, and 32 mg. was lethal for 2 of 5 animals (Table I).

TABLE I

The Lethal Effect of Liquoid Alone

Group	Amount of Liquoid	Number of rabbits	Number dying in less than 12 hours
	mg.		
Small rabbits	4	12	0
	8	8	2
	16	10	3
	32	14	9
Large rabbits	16	5	1
	32	5	2

Endotoxin and Liquoid. The enhancement of the lethal effect of Liquoid by meningococcal toxin is shown in Table II. Seventy per cent of the small rabbits given 8 mg. of Liquoid 12 hours after an injection of endotoxin and 100 per cent of those given 8 mg. of the

TABLE II

The Lethal Effect of Liquoid in Conjunction with Meningococcal Toxin

Group	Dilution of toxin	Dose of Liquoid	Interval between injections	Number of rabbits	Number dying in less than 12 hours
		mg.	hrs.		
Small rabbits	1:80	4	18	3	0
			24	12	0
		8	6	8	8
			12	7	5
			18	22	2
			24	9	1
			30	3	0
	1:1000	8	Simultaneous	4	1
Large rabbits	1:1000	16	Simultaneous	5	4
		32	Simultaneous	5	4

material 6 hours after endotoxin died within 12 hours. A similar effect was noted in the large animals which were given minute amounts of endotoxin simultaneously with Liquoid; 80 per cent of these died within 12 hours after the injection. Further data concerning the minimum effective doses of endotoxin are contained in another report.[13]

Morphologic Changes

The structural changes following the administration of Liquoid and Liquoid in conjunction with endotoxin are summarized in Tables III and IV.

TABLE III

Structural Changes Following the Injection of Liquoid Alone

Group	Dose of Liquoid	Number of rabbits	Renal lesion	Cardiac lesions	Cardiac fibrinoid	Splenic fibrinoid	Liver thrombi and necrosis	Pulmonary thrombi and hemorrhage	Adrenal hemorrhage
	mg.								
	4	12	0	4	0	0	0	0	0
Small	8	8	1	4	1	0	0	4	0
rabbits	16	10	8	4	3	2	4	10	1
	32	14	9	3	0	0	2	14	1
Large	16	5	0	0	0	0	0	2	0
rabbits	32	5	4	0	1	3	1	5	4

TABLE IV

Structural Changes Following the Injection of Liquoid in Conjunction with Meningococcal Toxin

Group	Dilution of toxin	Dose of Liquoid	Interval between injections	Number of rabbits	Renal lesion	Cardiac lesions	Cardiac fibrinoid	Splenic fibrinoid	Liver thrombi and necrosis	Pulmonary thrombi and hemorrhage	Adrenal hemorrhage
		mg.	*hrs.*								
		4	18	3	3	1	3	2	1	3	0
			24	12	0	8	2	3	2	4	0
			6	8	1	4	2	7	1	8	0
Small	1:80		12	7	3	2	2	6	1	6	1
rabbits		8	18	22	14	15	14	15	6	14	0
			24	9	4	7	3	5	3	7	1
			30	3	0	1	0	0	0	1	0
	1:1000	8	Simultaneous	4	3	2	1	1	0	4	1
Large		16	Simultaneous	5	5	2	2	3	2	5	4
rabbits	1:1000	32	Simultaneous	5	5	0	1	2	0	5	2

Kidney. The renal lesion resembled closely that produced by two injections of endotoxin (the generalized Shwartzman reaction). It

consisted of the presence of homogeneous, eosinophilic, refractile material within the lumina of the glomerular capillaries (Fig. 1), which usually was associated with tubular necrosis and interstitial hemorrhage. The material had the tinctorial features of fibrinoid, being purple-red with the Schiff stain, homogeneous purple to orange-purple with phosphotungstic acid-hematoxylin, reddish blue with crystal violet, yellow with van Gieson's stain, and a homogeneous blue with toluidine blue. In some animals which died within a short period, the material appeared somewhat more basophilic with hematoxylin, and was not closely applied to the capillary basement membranes.

In 85 per cent of the rabbits in which this material was present in the capillaries, gross cortical necrosis was observed. In occasional animals, only isolated areas of cortical infarction were noted, while in others the major portion of the kidney was necrotic (Fig. 8). In the latter, it was sometimes possible to demonstrate thrombi in the larger renal vessels. Saline suspensions of fresh glomeruli were prepared in the manner previously described[2] and examined in polarized light. It was found that the capillary material was intensely birefringent and had properties similar to the material which occurs in the glomeruli after two intravenous injections of endotoxin.

Heart. The cardiac lesions consisted of areas of muscle necrosis and mononuclear cellular reaction, muscle calcification, intravalvular hemorrhages, and coronary thrombi. The latter often were associated with typical areas of ischemic necrosis. Edema and vacuolization of the intima and media of the coronary arteries, similar to the lesion observed after a single injection of endotoxin,[1] were noted occasionally in the animals given only Liquoid, and were consistently observed in those given endotoxin and Liquoid. Hemorrhages in the myocardium were of such common occurrence, especially in those animals given large amounts of Liquoid, that they were not tabulated.

Fibrinoid also was found in the hearts of the animals given either Liquoid alone or endotoxin and Liquoid. In the animals given only Liquoid, however, it lay free in the pericardium or beneath the atrial endocardium, while in the animals given endotoxin in conjunction with Liquoid it appeared as a layer of acidophilic, refractile material lying beneath the endothelium of the coronary arteries and within the heart valves (Figs. 2, 4, and 5). The incidence of fibrinoid in the hearts of the animals given only Liquoid was low, comparable to that observed after a single intravenous injection of endotoxin.[1] When endotoxin was given in conjunction with Liquoid, the highest incidence

of cardiac fibrinoid occurred when the interval between injections was 18 hours (Table IV).

Spleen. The splenic lesion was similar to that observed after two injections of meningococcal toxin.[2] It consisted of the accumulation of material within the splenic sinusoids which, like the material in the glomerular capillaries, had the morphologic and tinctorial features of fibrinoid. Its accumulation was sufficient in many instances to produce areas of ischemic necrosis which had the gross appearance of infarcts.

Liver. The changes in the liver consisted of areas of hemorrhage and necrosis, associated with the presence of acidophilic, slightly granular, thrombotic material in the efferent veins (Fig. 3). Occasionally only perivascular hemorrhages were observed, but in 2 animals these were associated with the presence of fibrinoid beneath the endothelium of branches of the hepatic artery. In many instances, especially in those animals in which there was a short interval between the injections of toxin and Liquoid, no thrombi were observed, but the sinusoids contained material which resembled that seen in the splenic sinusoids and renal glomerular capillaries.

Lungs. Pulmonary thrombi or emboli, edema, and hemorrhage were consistently noted in the animals given large amounts of Liquoid alone, or in those in which there was a short interval between the injections of toxin and Liquoid. The thrombi or emboli were for the most part confined to the capillaries, but in those given toxin in addition to Liquoid they were observed also in large branches of the pulmonary arteries (Fig. 9). The thrombi, or emboli, were often homogeneous and deeply eosinophilic, and were purple-red when stained by the periodic acid-Schiff method.

Adrenal Gland. Adrenal hemorrhage was noted in only a small percentage of the animals. It occurred with equal frequency in animals given either Liquoid or endotoxin and Liquoid, except in the group of large rabbits. In these animals it was observed more often in the group given simultaneous injections of toxin and Liquoid. Most cases showed diffuse hemorrhage which replaced varying portions of the gland, but in one animal there were multiple infarcts in both adrenal glands (Fig. 10).

Brain. Changes in the central nervous system were observed only in those animals given endotoxin in association with Liquoid. The cerebral lesion consisted of the presence of material within the capillaries of the choroid plexus which was strikingly similar to that observed in the renal glomerular capillaries. Occasionally it appeared to lie beneath the endothelium, but in most cases it lay free in the lumen. It

usually was not applied closely to the basement membranes and in this respect differed from the renal glomerular material (Fig. 6). Its staining reactions, however, were similar to those of the material in the kidneys and spleens. Thrombi within the cerebral vessels also were noted occasionally (Fig. 11), in one instance lying in close proximity to an area of acute encephalomalacia.

Ears. Lesions involving the ears were observed only in those animals given endotoxin in association with Liquoid, particularly in those in which the interval between endotoxin and Liquoid was 18 hours. These changes occurred approximately 6 to 8 hours after the injection of Liquoid and increased in severity until death or sacrifice. Involvement of the ears was almost invariably associated with the development of bilateral renal cortical necrosis.

Grossly, the ears became boggy, purple-blue, and drooped. Microscopically, many of the vessels were occluded by dense masses of homogeneous, eosinophilic material resembling fibrinoid which replaced the wall of the vessel and extended into the perivascular spaces. This was associated with marked hemorrhage, edema, and varying degrees of cellular infiltration (Fig 7).

Other Organs. Hemorrhage within the thymus was observed in animals given large amounts of Liquoid and in those given endotoxin in association with smaller amounts of Liquoid. In many instances the entire gland appeared as a hemorrhagic mass of tissue. Intestinal hemorrhage was observed in 3 animals, 2 of which had been given endotoxin and Liquoid. In those latter animals many of the vessels in the hemorrhagic area were occluded by thrombi, and fibrinoid was present beneath the endothelium of the vessels.

The Effects of Dextran Sulfate and PVAS

The lethal effect of dextran sulfate and PVAS was not as marked as that observed with comparable amounts of Liquoid. None of the animals given 50 mg. of PVAS, for example, died within 12 hours. When given in conjunction with endotoxin, however, small amounts of these materials showed a pronounced lethal effect. Fifty per cent of the animals given 8 mg. of PVAS 6 hours after endotoxin died within 12 hours, and 40 per cent of those given a simultaneous injection of 10 mg. of dextran sulfate and endotoxin died within the same period. Further observations concerning the lethal effect of these substances in varying amounts and with varying dilutions of endotoxin will be documented elsewhere.[18]

The structural changes observed in these animals were similar to those described for the previous groups. No adrenal lesions were noted

in any of the animals, however, and no renal or splenic lesions developed in the animals given PVAS or dextran sulfate alone. The latter two lesions, however, developed with regularity when these polymers were given simultaneously with endotoxin (Tables V and VI). The material which accumulated in the renal glomerular capillaries and in the splenic sinusoids was similar to that observed in the Liquoid group, and had similar staining properties.

TABLE V

Structural Changes Following the Injection of PVAS Alone and in Conjunction with Meningococcal Toxin *

Dilution of toxin	Interval between injections	Number of rabbits	Dose of PVAS	Renal lesion	Cardiac lesions	Cardiac fibrinoid	Splenic fibrinoid	Hepatic thrombi and necrosis	Pulmonary thrombi and hemorrhage
	hrs.		*mg.*						
		4	8	0	2	0	0	0	2
None		4	16	0	3	1	0	1	4
		4	32	0	2	0	0	0	3
		4	50	0	2	0	0	3	2
1:1000	Simultaneous	4	8	3	2	1	3	1	4
1:80	6	4	8	2	3	1	4	1	2
	18	4	8	0	4	2	2	1	3

* All animals in these groups weighed 1 to 1.5 kg.

TABLE VI

Structural Changes Following the Injection of Dextran Sulfate Alone and in Conjunction with Meningococcal Toxin *

Dilution of toxin	Interval between injections	Number of rabbits	Dose of dextran sulfate	Renal lesions	Cardiac lesions	Cardiac fibrinoid	Splenic fibrinoid	Hepatic thrombi and necrosis	Pulmonary thrombi and hemorrhage
			mg.						
None		4	10	0	2	0	0	0	0
1:500	Simultaneous	10	10	9	5	0	6	5	5

* All animals in these groups weighed 1 to 1.5 kg.

DISCUSSION

The most characteristic gross lesion of the generalized Shwartzman phenomenon is bilateral renal cortical necrosis. This reaction occurs after two appropriately spaced intravenous injections of meningococcal endotoxin and does not take place, except under unusual circumstances,

after a single injection.[2,14] This lesion appears to develop as the result of occlusion of the glomerular capillaries by homogeneous material with the tinctorial features of fibrinoid.

In the present study it has been shown that three acidic polymers of large molecular size, possessing anticoagulant properties somewhat similar to heparin, are capable of producing an equivalent renal lesion when administered intravenously to rabbits previously or simultaneously given a single intravenous injection of endotoxin. In the case of Liquoid, the polymer alone can produce the renal lesion when given in sufficiently large amounts. Walton[4] has shown that dextran sulfate alone can produce a similar renal lesion when given in doses of 100 mg. per kg. With the material used in the present study, no renal changes occurred with doses as high as 50 mg. per kg., but larger amounts could not be tested because of the immediate lethal effect.[13] PVAS, administered alone in doses as high as 50 mg., produced no renal lesions.

Other lesions produced by two injections of endotoxin, discussed in the previous paper,[2] include accumulations of fibrinoid in the vessels and valves of the heart and the splenic sinusoids, and pulmonary and hepatic thrombi with areas of hemorrhage and necrosis. Entirely similar lesions were produced by the acidic polymers in animals given one injection of endotoxin and, in the case of Liquoid, by the polymer alone when given in sufficient dosages. It should be noted, however, that the incidence of fibrinoid in the coronary arteries and heart valves was considerably higher in the animals given endotoxin and Liquoid than with Liquoid alone, even when large amounts of the latter were given. It is of interest, also, that in the animals given endotoxin and Liquoid, the greatest incidence of cardiac fibrinoid occurred when there was an interval of 18 hours between the injections. Similarly, the splenic lesion occurred more frequently in those animals given both endotoxin and Liquoid than in those given Liquoid alone.

These differences in the incidence of various lesions emphasize the importance of endotoxin in "preparing" the animals. Its importance is shown also by the occurrence of cerebral and aural lesions in animals given endotoxin in addition to Liquoid. These observations suggest that certain changes occur following the administration of endotoxin which bring about localization of the fibrinoid in certain sites, and that an optimal interval exists for the administration of these polymers after an injection of toxin. The structural changes which occur after a single intravenous injection of endotoxin[1] may be important factors in the localization of this material.

In addition to structural changes, the possibility that endotoxin may in some way bring about further polymerization of the injected compound or that it may act to alter a compound in the circulating blood which then combines with the injected polymer should be considered also. The lethal effects of Liquoid, PVAS, and dextran sulfate are enhanced by a previous or simultaneous injection of endotoxin. Patat and Vogler[11] reported an increase in the toxicity of PVAS with increasing degrees of polymerization, and a similar observation was made by Walton[4] concerning the dextran sulfates.

Experimental observations, reported elsewhere,[16] have shown that a heparin-precipitable protein, apparently related to fibrinogen, appears in the blood of rabbits within 1 hour after an injection of endotoxin. Moreover, an injection of Liquoid at that time is followed by a precipitous drop in the amount of this heparin-precipitable protein, accompanied by an equally abrupt fall in the level of total blood fibrinogen. In this regard, the demonstration by Walton[4] of the *in vitro* precipitation of fibrinogen by dextran sulfate is of special interest. These observations suggest that fibrinogen may be altered by endotoxin, and that this alteration may constitute the most important "preparing" function of endotoxin.

The basic similarity between the changes observed in rabbits given two appropriately spaced injections of endotoxin and in those given endotoxin in association with Liquoid, PVAS, or dextran sulfate suggests that similar mechanisms are involved in the production of the lesions. The diffuse nature of the tissue lesions and the histologic appearance of the material occluding the blood vessels suggest some change in the circulating blood leading to vascular occlusion in various organs with subsequent ischemic necrosis. The demonstration that the lesions can be prevented by heparin[13] is further evidence in favor of the hypothesis that the reaction represents primarily a disturbance in the circulating blood. These features, and the experimental observations concerning fibrinogen, support the hypothesis that fibrinoid is derived from the circulating blood and that fibrinogen may play an important rôle in its formation.

Summary and Conclusions

Hybrid albino rabbits of mixed sexes were given an intravenous injection of sodium polyanetholsulfonate (Liquoid), dextran sulfate, or the sodium salt of polyvinyl alcohol polysulfonic acid ester (PVAS) in varying amounts, alone and in conjunction with an intravenous injection of meningococcal toxin.

A pronounced lethal effect was noted when Liquoid was given alone in large doses, or when given in small doses in association with meningococcal toxin. Comparable amounts of PVAS or dextran sulfate were relatively non-toxic when given alone, but when administered in association with endotoxin caused death in a majority of the animals.

Structural changes in the kidneys, heart, spleen, liver, adrenal glands, lungs, brain, and ears were noted in the animals given the acidic polymers in conjunction with endotoxin. The important histologic feature of the lesions was the presence of fibrinoid. This material was observed in the glomerular capillaries, pericardium, coronary arteries, heart valves, the splenic sinusoids, the capillaries of the choroid plexus, and in the vessels and perivascular spaces of the ears. In 85 per cent of the cases in which this material was present in the renal glomerular capillaries, gross cortical necrosis similar to that occurring in the generalized Shwartzman phenomenon was present. An interval of 18 hours between the injection of endotoxin and Liquoid appeared to be the optimal time for the development of cardiac fibrinoid lesions.

Hepatic thrombi and necrosis were observed with equal frequency in animals given large amounts of Liquoid and in those given endotoxin and smaller amounts of Liquoid. Bilateral adrenal hemorrhage and necrosis were observed in a small percentage of animals. Pulmonary hemorrhages and thrombi or emboli were an almost constant finding in the animals given large doses of Liquoid, and in those given a simultaneous injection of endotoxin and small amounts of Liquoid. Splenic and hepatic lesions were not produced by dextran sulfate or PVAS alone, but occurred with regularity when these were given in conjunction with endotoxin.

There is a basic similarity between the lesions observed in these animals and in those given two appropriately spaced intravenous injections of endotoxin (the generalized Shwartzman phenomenon). The morphologic features of the lesions, the widespread occurrence of fibrinoid, and the biologic activities of these acidic polymers with respect to fibrinogen support the hypothesis that fibrinoid is derived from the circulating blood and suggest that fibrinogen may be directly involved in its formation.

The photographs were made by Mr. Henry Morris.

REFERENCES

1. Brunson, J. G.; Gamble, C. N., and Thomas, L. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. I. The effects produced in young and in mature animals by a single injection. *Am. J. Path.*, 1955, 31, 489–499.

2. Brunson, J. G.; Thomas, L., and Gamble, C. N. Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the rôle of fibrinoid in the generalized Shwartzman reaction. *Am. J. Path.*, 1955, 31, 655–667.

3. Hausman, R., and Dreyfus, P. M. Intracapillary precipitates produced in rabbits by means of sodium polyanetholsulfonate. *A. M. A. Arch. Path.*, 1953, 56, 597–606.

4. Walton, K. W. Investigation of the toxicity of a series of Dextran sulfates of varying molecular weight. *Brit. J. Pharmacol.*, 1954, 9, 1–14.

5. Inderbitzin, T. Experimentelle Erzeugung von Lupus-erythematodes-Zellen. *Dermatologica*, 1952, 104, 222–226.

6. Inderbitzin, T. The experimental production of leukocytic changes in normal blood similar to LE-cells and morphologically identical with LE-cells. *J. Invest. Dermat.*, 1953, 20, 67–79.

7. von Haebler, T., and Miles, A. A. The action of sodium polyanetholsulphonate ("Liquoid") on blood cultures. *J. Path. & Bact.*, 1938, 46, 245–252.

8. Fitch, C. P.; Bishop, L. M., and Kelly, M. D. The isolation of *Brucella abortus* from the blood-stream of cattle. *Proc. Soc. Exper. Biol. & Med.*, 1936, 34, 696–698.

9. Inderbitzin, T. Compendium, Roche: herausgegeben von der Firma Hoffmann-LaRoche & Cie, A. G. Basel, Switzerland, 1948, pp. 168 and 223.

10. Demole, V., and Reinert, M. Polyanetholsulfosaures Natrium, ein neues synthetisches Mittel zur Hemmung der Blutgerinnung. *Arch. f. exper. Path. u. Pharmakol.*, 1930, 158, 211–218.

11. Patat, F., and Vogler, K. Polyvinylalkoholpolyschwefelsaureester als Modellsubstanzen fur synthetische Heparine. *Helv. Chim. Acta*, 1952, 35, 128–138.

12. Ricketts, C. R., and Walton, K. W. Dextran sulphate: the relation of molecular features to biological properties. *Brit. J. Pharmacol.*, 1953, 8, 476–477.

13. Thomas, L.; Brunson, J. G., and Smith, R. T. Studies on the generalized Shwartzman reaction. VI. Production of the reaction by the synergistic action of endotoxin with three synthetic acidic polymers (sodium polyanetholsulfonate—"Liquoid," dextran sulfate and sodium polyvinyl alcohol sulfate). *J. Exper. Med.* (In press.)

14. Thomas, L., and Good, R. A. Studies on the generalized Shwartzman reaction. I. General observations concerning the phenomenon. *J. Exper. Med.*, 1952, 96, 605–624.

15. Sevringhaus, E. Personal communication.

16. Thomas, L.; Smith, R. T., and Von Korff, R. Cold-precipitation by heparin of a protein in rabbit and human plasma. *Proc. Soc. Exper. Biol. & Med.*, 1954, 86, 813–818.

[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Renal glomerulus showing accumulation of homogeneous, eosinophilic material in the capillary loops. From a rabbit given 8 mg. of Liquoid 18 hours after an intravenous injection of meningococcal toxin and killed 24 hours later. × 450.

FIG. 2. Coronary artery with segmental deposition of fibrinoid in the media. Of note is the abrupt loss of nuclei. Rabbit given 4 mg. of Liquoid 24 hours after an injection of meningococcal toxin and killed in 24 hours. × 450.

FIG. 3. Section from the liver of rabbit given 8 mg. of Liquoid 24 hours after an injection of meningococcal toxin and killed in 24 hours. Of note is an extensive area of hemorrhage and necrosis. × 100.

FIG. 4. Coronary artery showing extensive subendothelial deposition of fibrinoid. Rabbit given 4 mg. of Liquoid 18 hours after an injection of meningococcal toxin; killed in 30 hours. × 450.

FIG. 5. Cardiac valve leaflet showing extensive hemorrhage and a minimal deposition of fibrinoid. Rabbit given 8 mg. of Liquoid 12 hours after an injection of meningococcal toxin. Killed in 30 hours. × 100.

FIG. 6. Section from the choroid plexus of a rabbit given 8 mg. of Liquoid 18 hours after an injection of meningococcal toxin. The accumulation of material in these capillaries is similar to that in the glomerular capillaries. In the lower vessel it appears to be beneath the endothelium but otherwise it is intraluminal. Animal killed 30 hours after the injection of Liquoid. × 250.

FIG. 7. Section from the ear of a rabbit given 8 mg. of Liquoid 18 hours after an injection of meningococcal toxin and killed 30 hours later. There is extensive hemorrhage and most of the media of the artery is replaced by fibrinoid. Similar material is present also in the lumen of the vessel. × 250.

1

3

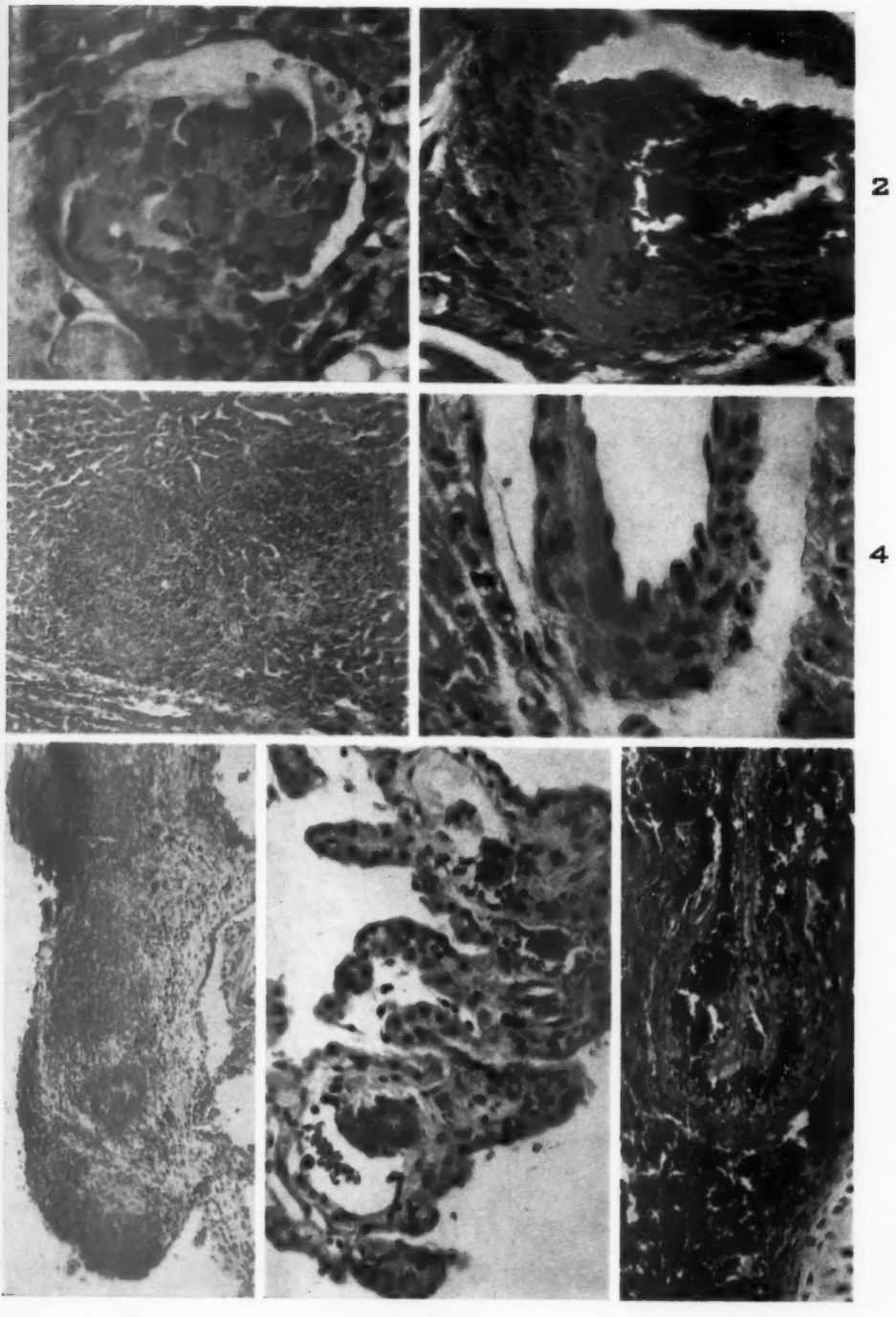

1
2
3
4
5
6
7

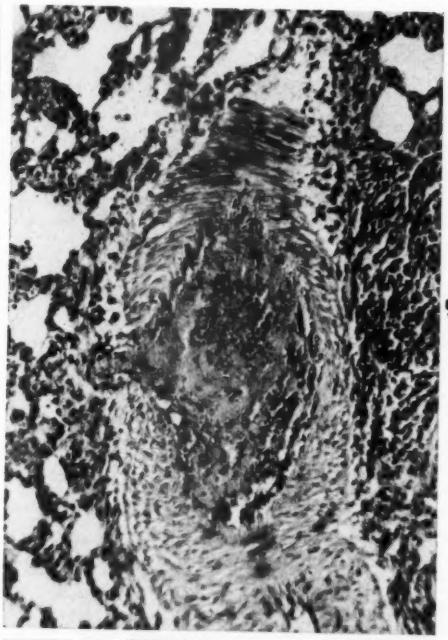
FIG. 8. Gross appearance of the kidney from a rabbit given a simultaneous intravenous injection of meningococcal toxin (dilution 1:1000) and 8 mg. of Liquoid. The animal died within 24 hours. Extensive hemorrhage and necrosis involve both cortex and medulla. Formalin fixation.

FIG. 9. Section from the lung of rabbit given 8 mg. of Liquoid 18 hours after an intravenous injection of meningococcal toxin and killed 30 hours later. There is a thrombus or embolus in a large branch of the pulmonary artery. × 150.

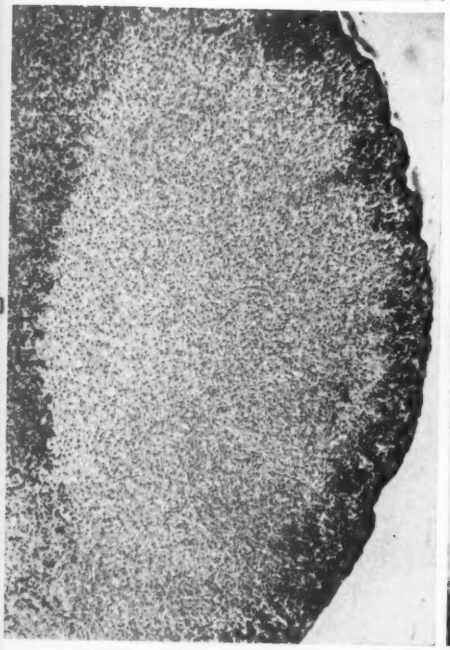
FIG. 10. Section from the adrenal gland of a mature rabbit given 32 mg. of Liquoid and killed in 24 hours. Of note is an extensive area of infarction. × 65.

FIG. 11. Cerebral vessel showing the presence of a thrombus with numbers of enmeshed cells and nuclear débris. Rabbit given 8 mg. of Liquoid 18 hours after an injection of meningococcal toxin; killed in 30 hours. × 250.

8



9



10

11

ANALYTICAL PATHOLOGY

II. Histopathologic Demonstration of Glomerular-Localizing Antibodies in Experimental Glomerulonephritis *

Robert C. Mellors, M.D.; Javier Arias-Stella, M.D.†; Malcolm Siegel, Ph.D., and David Pressman, Ph.D.‡

From the Divisions of Pathology (Drs. Mellors and Arias-Stella) and Experimental Pathology (Drs. Siegel and Pressman), Sloan-Kettering Institute for Cancer Research; the Pathology Laboratories (Drs. Mellors and Arias-Stella), Memorial Center for Cancer and Allied Diseases; and the Sloan-Kettering Division, Cornell University Medical College (Drs. Mellors and Pressman), New York, N.Y.

In a previous communication,[1] a method—in reality a new application of the technique of Coons and Kaplan[2] for the detection of antigens—was described for the histologic demonstration of the antigenic components of tissues and the sites of *in vivo* localization of antibodies. Aside from showing that the glomeruli and particularly their basement membranes were the principal renal antigens in the production of nephrotoxic serum, the method disclosed that antibodies localize in glomeruli during the acute stage of experimental glomerulonephritis produced in rabbits by the injection of purified foreign proteins, namely, bovine gamma globulins. The main purpose of the present paper is to describe in fuller detail the experimental conditions and observations pertaining to the rôle of glomerular-localizing antibodies in the pathogenesis of experimental glomerulonephritis. In addition, studies were made of three other histopathologic manifestations of allergy and hypersensitivity, namely, myocarditis, endocarditis, and angiitis. Since it is necessary to document the anatomical changes observed, a histopathologic description of the lesions occurring in the experimental animals is presented.

Materials and Methods

Experimental Procedure

Young, male, white rabbits with an average weight of 2,400 gm. were used. Fifteen animals received intravenous injections of bovine gamma globulins, while 4 served as normal control animals.

* This investigation was supported in part by research grant C–1345 from the National Cancer Institute of the National Institutes of Health, Public Health Service; by a grant from the Damon Runyon Memorial Fund; and by the United States Atomic Energy Commission under their contract A T (30–1) — 910.

Received for publication, November 5, 1954.

Presented at the Fifty-second Annual Meeting of the American Association of Pathologists and Bacteriologists, Houston, Texas, April 8, 1955.

† Kellogg Foundation Fellow in Pathology. Now at Faculty of Medicine, Lima, Peru, South America.

‡ Now at Roswell Park Memorial Institute, Buffalo, N.Y.

The bovine gamma globulins (Armour & Company, Fraction II) were neutralized, made up in physiologic sodium chloride solution, and sterilized, as proved with bacteriologic cultures, by pressure-filtration through a Seitz filter. The final concentration of protein was 8 gm. per 100 ml.; and this solution was given intravenously in a marginal ear vein at a dose of 10 ml. per kg. of body weight, administered once a week. As shown in Table I, 7 animals received one injection while the remainder received two to three injections. A small desensitizing dose (about 1 ml. per kg. of 1 per cent protein solution) was given intravenously about 6 to 12 hours prior to the second and third injections in order to prevent anaphylactic shock (which nevertheless occurred in rabbit S). In addition to receiving bovine gamma globulins, 4 animals (Table I) were subjected to unilateral nephrectomy with the intent of increasing the frequency or severity of the renal lesions.[3]

TABLE I

Number of Intravenous Doses of Bovine Gamma Globulins (BGG) and Other Treatment Received by Rabbits

Animals	Number of doses of BGG*	Other treatment
B, C, E, F, G	1	
I, J	1	Unilateral nephrectomy
A, H, S	2	
R, T	2½	
L	2½	Unilateral nephrectomy
Q	3	
N	3	Unilateral nephrectomy

* Each dose consisted of the intravenous injection of 10 ml. per kg. of body weight of 8 per cent bovine gamma globulins made up in sterile isotonic saline solution.

Animals were weighed daily, and they displayed in some cases a terminal weight loss. Twenty-four hour urine collections were made by placing the animals in metabolism cages on one or more occasions weekly. The urine was examined microscopically; and the protein concentration was estimated by the Kingsbury-Clark method.[4] When the quantity of protein was 20 mg. per 100 ml. of urine, or greater, on more than one occasion—and the finding was frequently verified by analysis of the bladder urine at necropsy—the result was recorded as positive for proteinuria.

The animals were sacrificed on or about the seventh day after the last injection of protein. They were anesthetized with sodium phenobarbital, given heparin to prevent blood coagulation, and perfused with isotonic saline solution under a hydrostatic pressure of 2.5 m. until the

abdominal and the thoracic viscera were grossly cleared of blood. The perfusion fluid entered the abdominal aorta in a cephalic direction and passed through the transected inferior vena cava. Bladder urine was taken for protein and microscopic analysis and on occasion for bacteriologic culture. The kidneys, liver, spleen, heart, and lungs were weighed (Tables II and III) and representative blocks were cut for

TABLE II

Weights of Perfused Organs in Normal Control Rabbits

Body weight	Organ weights as per cent of body weight				
	Kidney	Heart	Lung	Spleen	Liver
AV 2420	0.45	0.36	0.25	0.076	3.8
CI 2210–2630	0.39–0.51	0.31–0.41	0.19–0.31	0.046–0.106	2.4–5.2

AV = average; CI = confidence interval that statistically includes 99 per cent of the estimates of the average.

TABLE III

Weights of Perfused Organs in Rabbits Receiving Injections of Bovine Gamma Globulins

Animal	Body weight	Organ weights as per cent of body weight						
		Rt. kidney	Lt. kidney	Heart	Rt. lung	Lt. lung	Spleen	Liver
	gm.							
A	2690	0.36	0.38	0.28	0.23	0.17	0.074	4.1
B	2440	0.50	0.49	0.31	0.27	0.20	0.074	4.8
C	2100	0.49	0.53	0.45	0.26	0.20	0.11	4.5
E	2300	0.38	0.37	0.28	0.28	0.22	0.096	4.8
F	2640	0.44	0.49	0.38	0.23	0.20	0.076	5.7
G	3630	0.38	0.38	0.34	0.41	0.27	0.099	4.8
H	2440	0.42	0.52	0.37	0.27	0.19	0.11	3.8
I	1790	0.56	0.39*	0.42	0.25	0.18	0.10	5.3
J	2080	0.52	0.48*	0.41	0.26	0.19	0.13	3.8
L	2320	0.66	0.41*	0.50	0.21	0.18	0.13	2.8
N	1980	0.82	0.33*	0.41	0.45	0.31	0.14	6.3
Q	2830	0.43	0.51	0.25	0.27	0.18	0.10	4.8
R	2810	0.51	0.58	0.34	0.56	0.40	0.06	4.7
S	2270	0.50	0.52	0.31	0.34	0.28	0.18	4.5
T	2040	0.56	0.45	0.29	0.25	0.17	0.12	3.5

* Unperfused, normal kidney obtained by nephrectomy before injections of bovine gamma globulins were begun.

use in the following manner. Unfixed tissue for the study of antibody localization was frozen immediately on dry ice and stored at −10° C. Frozen sections, 3 to 5 μ thick, were subsequently prepared from this material by the method of Bush and Hewitt[5] and treated with the

fluorochroming procedure to be described. Blocks for routine histologic observation were fixed in 10 per cent formalin at pH 7.0. Paraffin sections were stained with Harris' hematoxylin and picric acid eosin and the periodic acid-Schiff reagent. When indicated, special stains such as scharlach R, Weigert's resorcin-fuchsin, Mallory's anilin blue, and colloidal iron[6] were used.

Demonstration of Localizing Antibodies

The method has been described in full elsewhere[1]; and similar procedures were used for the preparation of the globulin fraction from chicken antiserum to rabbit globulins, designated herein as Chi-anti-Rab, and for the labelling or coupling of this fraction with fluorescein isocyanate to obtain the fluorochroming reagent, designated herein as the fluor. When an unfixed, thin frozen section of rabbit tissue is washed with pH 8 isotonic borate buffer for 1 minute, incubated with a few drops of the fluor for 1 hour at room temperature, washed with borate buffer, rinsed and mounted in buffered glycerin, and examined with a fluorescence microscope, rabbit antibodies that are localized in the tissue become self-luminous because of their combination with the fluor. The immunochemical specificity of the fluorochroming procedure is verified[2] by demonstrating that either a decreased intensity or a total absence of fluorescence occurs when the tissue section is incubated with uncoupled Chi-anti-Rab prior to treatment with the fluor. All observations pertaining to antibody localization in the present study were verified by this demonstration. The selectivity of the procedure was enhanced also[2] by treatment of the fluorochroming reagent with lyophilized tissue protein. In some of the present work, the washed and sedimented fraction of homogenized rabbit liver was used for this purpose.

The method and the conditions of photographic photometry which permit a translation of the photographic density into the relative fluorescence intensity of histologic details are similar to those previously used.[1]

Results

Histopathologic Findings

The significant microscopic findings in the organs of rabbits receiving intravenous injections of bovine gamma globulins are given in Table IV. The classification and the incidence of lesions in 15 treated rabbits were as follows: glomerulonephritis, 12; myocarditis, 8; endocarditis, 3; interstitial pneumonia, 5; allergic angiitis, 4; lymphoid hyperplasia of the spleen, 15; allergic granuloma of the spleen, 2; and

parasites of the liver, 6. A brief description of the gross and the microscopic anatomy of the kidneys, heart, lungs, spleen, and liver in these animals follows.

TABLE IV

Incidence of Histopathologic Lesions and Alterations in Organs of Rabbits Receiving Bovine Gamma Globulins

Animal	Glomerulonephritis	Myocarditis	Endocarditis	Interstitial pneumonia	Angiitis	Lymphoid hyperplasia, spleen	Granuloma, spleen	Parasites liver
A	+	+	+	+		+		
B	+					+		
C	+	+	+			+		+
E	+					+	+	+
F	+	+				+		+
G	+		+	+	+	+	+	
H		+		+	+	+		
I						+		
J	+					+		
L	+	+				+		
N	+	+				+		
Q	+	+		+	+	+		+
R	+					+		+
S	+	+		+	+	+		
T						+		+
Incidence	12/15	8/15	3/15	5/15	4/15	15/15	2/15	6/15

The evidence for, and the incidence of, renal injury are given in Table V. Proteinuria occurred in 5 of 15 treated animals and by the criteria used was not present in the normal controls. Gross enlargement together with punctate hemorrhages in the superficial cortex occurred in the kidneys of one animal (R). Hypertrophy of the remaining kidney occurred in all animals having unilateral nephrectomy (data in Table III compared with those in Table II); and the average weight of the kidney (the sum of both divided by two) was increased in 2 nephrectomized animals (L and N). Microscopic findings of glomerular injury were present in 12 animals. The most frequent and diffuse lesion occurred in the glomerular capillaries and appertaining structures. The glomerular capillary change comprised three alterations which singly or collectively produced a partial or complete occlusion of the lumina of the capillary loops: (a) swelling or enlargement (and increased staining) of the cytoplasm of cells, a change which occurred principally in endothelial cells and to a lesser extent in epi-

thelial and mesenchymal cells; (b) widening of the intercapillary spaces and the presence therein of homogeneous and occasionally granular acidophilic material; and (c) alterations of the basement membranes consisting of thickening and the deposition of hyaline or fibrillar acidophilic material, a change which was more prominent in focal areas of the capillary tufts.

Cellular proliferation, mainly of endothelial origin, was the next most frequent evidence of glomerular injury. The lesion was focal in some

TABLE V

Evidence of Renal Injury in Rabbits Receiving Bovine Gamma Globulins

Animals	Proteinuria	Renal enlargement	Renal petechiae	Glomerulitis					Tubular casts
				Capillary change	Cellular proliferation	Cellular exudation	Focal necrosis	Crescent formation	
A				+					+
B				+	+			+	+
C				+					
E				+	+	+			+
F				+	+				+
G				+	+				
H									
I									
J				+					+
L	+	(+)		+	+				
N	+	(+)		+				+	+
Q	+			+	+		+	+	+
R	+	+	+	+	+			+	+
S	+			+	+				
T									

kidneys and diffuse in others, and in many glomeruli the proliferative change was confined principally or entirely to segmental or lobular portions of the capillary tufts. The endothelial proliferation completely occluded the capillary lumina in focal areas of some glomeruli. The increased number of polymorphonuclear leukocytes in the glomerular capillaries was noted in many cases and was clearly excessive in one animal (E). Foci of necrosis of glomeruli were present in one animal (Q), and the lesions were composed of fragmented nuclei of inflammatory and parenchymatous cells superimposed on hyalinized or structureless capillary walls. Crescent formation and adhesion between the capillary tuft and the glomerular capsule were present in 4 animals.

Protein casts in the glomerular space and in the convoluted tubules were of frequent occurrence, and red blood cells in various stages of degeneration were present in these locations on rare occasion. The walls of renal cortical arterioles and venules were thickened and hyalinized in several animals.

Interstitial pneumonia was present in 5 animals. The lesions were unevenly distributed within the lobe and among the lobes, were associated with allergic angiitis in all but one case, and were composed of large collections of mononuclear cells, including lymphocytes, monocytes, and plasma cells, together with a few neutrophils and eosinophils. The inflammatory exudate had an alveolar, septal, perivascular, and peribronchial distribution. Angiitis, when most extensive, was present in the arteries, arterioles, capillaries, venules, and veins of the lung and consisted of endarteritis, endophlebitis, and panvasculitis. The inflammatory exudate, which included mononuclear cells and serous fluid together with small numbers of neutrophils and eosinophils, was most prominent beneath the endothelium and in some cases was present in all coats of the vascular wall. In some blood vessels there was proliferation of endothelial lining cells with partial or total occlusion of the lumina. The cellular exudate of the interstitial pneumonitis appeared in some instances to arise by extension from the vascular inflammation.

Focal interstitial myocarditis was present in 8 of 15 treated animals. The lesions were composed of microscopic, nodular collections of mononuclear cells, principally Anitschkow myocytes but also lymphocytes and plasma cells, which had a predilection for distribution in the perivascular connective tissue of the myocardium. Necrosis of connective tissue as well as of muscle fibers frequently was present in the centers of the lesions.

Endocarditis, including mitral valvulitis, was present in 3 animals. The valvular lesions consisted of foci of edema and fibrinoid necrosis accompanied by exudation of mononuclear and polymorphonuclear cells and proliferation of cardiac histiocytes.

Hyperplasia of the lymphoid follicles of the spleen occurred in all of the treated animals. In two cases granulomas composed of nodular collections of epithelioid cells together with giant cells were present in the follicles of the spleen.

Coccidia were present in the livers, this being the only abnormal finding that was common to both the treated and the control animals.

Many of the histopathologic lesions are illustrated in Figures 1 to 17.

Localizing Antibodies

Representative photomicrographs of thin, unfixed, frozen sections of rabbit kidneys which were treated with the fluor (fluorescein-labelled Chi-anti-Rab) to demonstrate the localization of antibodies by fluorescence are shown in Figues 18 to 30; and the quantitative data are summarized in Table VI.

TABLE VI

Glomerular: Tubular Ratio of Fluorescence Intensity in Sections of Rabbit Kidneys Treated with the Fluorochroming Procedure for Demonstration of Localizing Antibodies

Group of animals	No. of animals	Glomerulitis	Ratio of fluorescence intensity		
			AV	CI	N
Control	2	Not present	1.4	1.3–1.5	52
Control+	6	Not present	1.4	1.3–1.5	94
Experimental	3	Not present	1.6	1.4–1.8	48
Experimental	3	Capillary change only	1.4	1.3–1.5	45
		Capillary change plus:			
Experimental	4	(a) cellular proliferation	2.0	1.9–2.2	69
Experimental	5	(b) proliferation, crescents, etc.	2.1*	2.0–2.3	101

AV = average.
CI = confidence interval which includes 99 per cent of the estimates of the average.
N = number of measurements.
Control = normal animals, perfused.
Control + = perfused and unperfused.
Experimental = injected with bovine gamma globulins.
* When the fluorochroming procedure is ***blocked*** by pretreatment of sections with Chi-anti-Rab, the values are: AV, 1.3; CI, 1.2-1.5, and N, 33, respectively.

The fluorescence in a section of normal kidney obtained from a control rabbit is due principally to non-specific combination between the histologic structures and the fluor. The fluorescence intensity of the glomerulus is slightly greater than that of many of the tubules, as judged by visual estimation (Figs. 18 and 19); and the glomerular: tubular ratio of fluorescence intensity, as measured photometrically, is 1.4 (Table VI).

In a kidney of a rabbit having glomerulitis with cellular proliferation, the fluorescence intensity of the glomeruli is much greater than that of the tubules (Figs. 20 to 23); and the analytical glomerular: tubular ratio of fluorescence intensity is 2.0 (Table VI). This is a statistically significant increase in the ratio relative to that for the normal control kidney, because, as shown in the fifth column of Table VI, the confidence intervals (lines 1 or 2 and 5) do not overlap. That the increased fluorescence intensity displayed in proliferative glomerulitis is due to the presence of glomerular-localizing antibodies is shown

as follows. If a section of kidney from a rabbit having proliferative glomerulitis is first treated with unlabelled globulin (Chi-anti-Rab) and then treated with the fluor, the fluorescence intensity of the glomerulus is then only slightly greater than that of the tubules (Figs. 24 and 25). The quantitative data (Table VI, last line and footnote) indicate that the glomerular:tubular ratio of fluorescence intensity is now 1.3, that is, essentially the same as the ratio in the control kidney (Table VI, lines 1 and 2). The explanation is that once the glomerular-localizing rabbit antibodies have combined with the unlabelled globulin, their further combination with the labelled globulin (the fluor) is prevented. The fluorescence which can be specifically blocked in this manner is due, therefore, to the presence of localizing antibodies which are concentrated in the glomerulus.

The glomerular:tubular ratio of fluorescence intensity is approximately 2.0 to 2.1 (Table VI, lines 5 and 6) in kidneys in which glomerulitis is accompanied by cellular proliferation (Figs. 20 to 23, and 28), cellular exudation (Fig. 27), or crescent formation (Fig. 26). However, if the glomerulitis consists only of those alterations in the glomerulus which were described as capillary changes in the discussion of histopathology, the glomerular:tubular ratio of fluorescence intensity is 1.4 (Table VI, line 4, and Fig. 29), which is indistinguishable from that in the normal control kidney.

If a rabbit, although receiving injections of foreign proteins, does not develop glomerulitis, the glomerular:tubular ratio of fluorescence intensity is 1.6 (Table VI, line 2, and Fig. 30), a value which is not statistically different from that in the normal control kidney.

In summary, antibodies, as demonstrated by the method of microfluorescence, are localized in the glomeruli of the kidneys of rabbits having glomerulitis of the proliferative, exudative, or crescentic type and induced by the injection of foreign proteins.

Preliminary studies have been made on other organs and tissues obtained from the animals in these experiments. The information at hand suggests that antibodies are localized in (a) the endothelium and subendothelium of pulmonary blood vessels that are the site of angiitis; (b) the endocardium and myocardium in carditis; and (c) the cytoplasm of plasma cells in the spleen of both normal and experimental animals. These findings will be the subject of a future report.

Discussion

The incidence, distribution, and types of glomerulitis and glomerulonephritis, as well as the associated allergic lesions, observed in the present work are similar to those described under comparable condi-

tions by other investigators (Table VII), notably, Rich and Gregory,[7] Hawn and Janeway,[8] Ehrich *et al.*,[9] More and Waugh,[8] Wissler *et al.*,[10] McLean *et al.*,[11] Hamilton-Paterson and Henderson,[12] Germuth,[13] and Hamilton and Fremes.[14]

The bearing that the present study has on the pathogenesis of glomerulonephritis must be viewed in historical perspective. The belief that human glomerulonephritis is of allergic origin has been expressed by clinicians, among them von Pirquet and Schick[15] and Volhard,[16] during the last half century. Fishberg,[17] in 1939, summarized the evidence in favor of the view that immuno-allergic mechanisms were in some way connected with the development of glomerulonephritis. The findings cited include: (a) the fact that glomerulonephritis complicating scarlatina or tonsillitis occurs during convalescence rather than at the height of the infection; (b) the not uncommon association of clinical glomerulonephritis with serum sickness; (c) the increased skin reactivity to filtrates of cultures of hemolytic streptococci shown by patients with glomerulonephritis; (d) an elevated titer of antibodies, including antistreptolysin, in the blood of patients with glomerulonephritis following scarlatina or pharyngitis; (e) the more frequent occurrence of glomerulonephritis in subacute bacterial endocarditis during the bacteria-free rather than the bacteremic stage of the disease; and (f) evidence from animal experiments which will be discussed.

Masugi,[18] in work reported during 1928 to 1934, produced experimental glomerulonephritis in rabbits by the injection of a specific antikidney serum. The clinical, chemical, and anatomical findings in the experimental animals corresponded closely to those in human diffuse glomerulonephritis; and the renal disease once established continued its progress and eventually produced death by renal insufficiency. Our recent findings with the microfluorescence method reported elsewhere,[1] together with the earlier work of others,[19-26] indicate that the glomeruli, and particularly their basement membranes, are the principal antigenic components that promote the formation of, and undergo combination with, the antibodies in antikidney serum.

Many investigators, since the early studies of Longcope,[27] have attempted to produce glomerulonephritis experimentally by sensitizing animals to foreign proteins, bacteria, and bacterial products. Some of this work has already been summarized in Table VII. Allen[28] stated that, of all the experimental procedures used, those employing injections of gamma globulins or antikidney antibodies produce histologic lesions which most closely simulate those of the spontaneous disease of human beings. To the foregoing evidence, all of which suggests an

TABLE VII

Glomerulitis, Glomerulonephritis, and Other Allergic Lesions Produced in Rabbits by Injections of Foreign Proteins

Reference	Material injected	Glomerulitis or glomerulonephritis			Other allergic lesions
		Incidence	Distribution	Principal lesion	
Rich & Gregory[7]	Horse serum	10/14	Diffuse	Proliferative	Periarteritis
Hawn & Janeway[8]	Bovine gamma globulin	11/19	Diffuse?	Proliferative and exudative	Carditis, arthritis, and arteritis
Ehrich *et al.*[9]	Horse serum	45/56	Diffuse	Proliferative, exudative, and necrotizing	Carditis, arteritis, and granulomas of spleen
More & Waugh[3]	Bovine gamma globulin	14/18	Diffuse	Proliferative	Carditis
Wissler *et al.*[10]	Horse serum	7/46	Focal	Necrotizing	Carditis and arteritis
McLean *et al.*[11]	Horse serum	20/31	Diffuse	Proliferative	None
Hamilton-Paterson & Henderson[12]	Horse serum	10/31	Diffuse	Membranous and proliferative	Periarteritis
Germuth[13]	Bovine albumin	28/34	Diffuse	Membranous and proliferative	Carditis and granulomas of spleen
Hamilton and Fremes[14]	Horse serum	28/51	Diffuse	Membranous and proliferative	Arteritis and carditis

underlying allergic pathogenesis of glomerulonephritis, is to be added the finding, first reported by Veil and Buchholz[29] and extended in the observations of others,[30,31] that the titer of serum complement is decreased in patients or animals with acute glomerulonephritis. This finding may have explanation in the work of Germuth,[18] who has shown in animals with induced hypersensitivity that the tissue lesions, among them glomerulonephritis, occur during the time when antigen is removed from the circulation following combination with circulating antibody.

The present study is the first to demonstrate that gamma globulin (presumably rabbit antibody to injected protein) is localized in the glomeruli in acute glomerulonephritis in the experimental animal. This localization of antibody, which is presumably due to a previous fixation in the glomeruli of some of the injected antigen, is an essential requisite for the allergic pathogenesis of glomerulonephritis. The analytical method used in this work should permit the study of the pathogenesis of other diseases, among them rheumatic fever and other so-called collagen diseases, rheumatoid arthritis, and certain types of purpura and encephalomyelitis. Gamma globulins have now been detected in the glomerular lesions in lipid nephrosis, acute glomerulonephritis, and secondary amyloidosis (Mellors and Ortega[32]).

Summary and Conclusions

The main purpose of this investigation was to study the rôle of tissue-localizing antibodies in the pathogenesis of experimental glomerulonephritis. The unique feature of the study was the histopathologic application of a microfluorescence method for demonstrating the histologic sites of localization of antibodies *in vivo*.

Of 15 rabbits receiving one or more intravenous injections of purified foreign proteins (bovine gamma globulins), 12 developed glomerulonephritis; 8, myocarditis; 3, endocarditis; 5, interstitial pneumonia; 4, allergic angiitis; 15, lymphoid hyperplasia of the spleen, and 2, allergic granulomas of the spleen.

The evidence of renal injury in 12 animals consisted variously of proteinuria, renal enlargement, renal petechiae, tubular casts, and glomerulitis with capillary change, cellular proliferation, cellular exudation, focal necrosis, and crescent formation.

Antibodies were localized in the glomeruli of the kidneys of rabbits having acute glomerulonephritis of the proliferative, exudative, or crescentic type. Such localization, not heretofore demonstrable, is clearly an essential requisite for the allergic pathogenesis of glomerulonephritis.

Preliminary studies of other organs in these sensitized animals suggested that antibodies were localized in the blood vessels in angiitis and in the heart in endocarditis and myocarditis.

The analytical method used should permit the study, already in progress, of the rôle of antigen-antibody interactions in the pathogenesis of a number of human diseases, among them, glomerulonephritis, rheumatic fever, disseminated lupus erythematosus, rheumatoid arthritis, thrombotic thrombocytopenic purpura, and certain types of encephalomyelitis.

We wish to thank Mr. John Hlinka and Miss Betty Corcoran for assistance.

REFERENCES

1. Mellors, R. C.; Siegel, M., and Pressman, D. Analytic pathology. I. Histochemical demonstration of antibody localization in tissues, with special reference to the antigenic components of kidney and lung. *Lab. Investigation*, 1955, 4, 69–89.
2. Coons, A. H., and Kaplan, M. H. Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. *J. Exper. Med.*, 1950, 91, 1–13.
3. More, R. H., and Waugh, D. Diffuse glomerulonephritis produced in rabbits by massive injections of bovine serum gamma globulin. *J. Exper. Med.*, 1949, 89, 541–554.
4. Kolmer, J. A.; Spaulding, E. H., and Robinson, H. W. Approved Laboratory Technic. Appleton-Century-Crofts, Inc., New York, 1951, ed. 5, p. 142.
5. Bush, V., and Hewitt, R. E. Frozen sectioning. A new and rapid method. *Am. J. Path.*, 1952, 28, 863–873.
6. Rinehart, J. F., and Abul-Haj, S. K. An improved method for histologic demonstration of acid mucopolysaccharides in tissues. *A. M. A. Arch. Path.*, 1951, 52, 189–194.
7. Rich, A. R., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, 72, 65–88.
8. Hawn, C. V. Z., and Janeway, C. A. Histological and serological sequences in experimental hypersensitivity. *J. Exper. Med.*, 1947, 85, 571–590.
9. Ehrich, W. E.; Forman, C. W., and Seifer, J. Diffuse glomerulonephritis and lipid nephrosis. Correlation of clinical, morphological, and experimental observations. *A. M. A. Arch. Path.*, 1952, 54, 463–503.
10. Wissler, R. W.; Smull, K., and Lesh, J. B. The effects of various horse serum fractions in producing cardiovascular and renal lesions in rabbits. *J. Exper. Med.*, 1949, 90, 577–594.
11. McLean, C. R.; Fitzgerald, J. D. L.; Younghusband, O. Z., and Hamilton, J. D. Diffuse glomerulonephritis induced in rabbits by small intravenous injections of horse serum. *A. M. A. Arch. Path.*, 1951, 51, 1–11.
12. Hamilton-Paterson, J. L., and Henderson, W. G. Serum nephritis in rabbits. *Brit. J. Exper. Path.*, 1952, 33, 67–75.
13. Germuth, F. G., Jr. A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J. Exper. Med.*, 1953, 97, 257–282.

UNIVERSITY of PENNSYLVANIA LIBRARIES

14. Hamilton, J. D., and Fremes, N. E. The natural history of experimental glomerulonephritis produced by foreign protein. *Am. J. Path.*, 1954, **30**, 127–145.

15. von Pirquet, C., and Schick, B. Die Serumkrankheiten. Franz Deuticke, Leipzig and Wien, 1905, pp. 60–68.

16. Volhard, F. Die doppelseitigen hämatogenen Nierenerkrankungen (Bright'sche Krankheit). Julius Springer, Berlin, 1918, pp. 358–472.

17. Fishberg, A. M. Hypertension and Nephritis. Lea & Febiger, Philadelphia, 1939, ed. 4, p. 448.

18. Masugi, M. Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum. Ein Beitrag zur Pathogenese der diffusen Glomerulonephritis. *Beitr. z. path. Anat. u. z. allg. Path.*, 1933–34, **92**, 429–466.

19. Greenspon, S. A., and Krakower, C. A. Direct evidence for the antigenicity of the glomeruli in the production of nephrotoxic serums. *Arch. Path.*, 1950, **49**, 291–297.

20. Heymann, W., and Lund, H. Z. Lipemic nephrosis in rats. *Science*, 1948, **108**, 448–449.

21. Krakower, C. A., and Greenspon, S. A. Localization of the nephrotoxic antigen within the isolated renal glomerulus. *A. M. A. Arch. Path.*, 1951, **51**, 629–639.

22. Pressman, D.; Eisen, H. N., and Fitzgerald, P. J. The zone of localization of antibodies. VI. The rate of localization of anti-mouse-kidney serum. *J. Immunol.*, 1950, **64**, 281–287.

23. Pressman, D.; Hill, R. F., and Foote, F. W. The zone of localization of anti-mouse-kidney serum as determined by radioautographs. *Science*, 1949, **109**, 65–66.

24. Pressman, D., and Keighley, G. The zone of activity of antibodies as determined by the use of radioactive tracers; the zone of activity of nephritoxic antikidney serum. *J. Immunol.*, 1948, **59**, 141–146.

25. Smadel, J. E. Experimental nephritis in rats induced by injection of anti-kidney serum. I. Preparation and immunological studies of nephrotoxin. *J. Exper. Med.*, 1936, **64**, 921–942.

26. Solomon, D. H.; Gardella, J. W.; Fanger, H.; Dethier, F. M., and Ferrebee, J. W. Nephrotoxic nephritis in rats. Evidence for the glomerular origin of the kidney antigen. *J. Exper. Med.*, 1949, **90**, 267–272.

27. Longcope, W. T. The production of experimental nephritis by repeated proteid intoxication. *J. Exper. Med.*, 1913, **18**, 678–703.

28. Allen, A. C. The Kidney. Grune & Stratton, New York, 1951, p. 114.

29. Veil, W. H., and Buchholz, B. Der Komplementschwund im Blute. Eine bedeutsame immunobiologische Reaktion beim rheumatischen Infekt und ihre Beziehung zur allgemeinen Pathologie des Rheumatismus. *Klin. Wchnschr.*, 1932, **11**, 2019–2021. (Cited by Masugi.[18])

30. Lange, K.; Slobody, L.; Graig, F.; Ogur, G.; Oberman, J., and LoCasto, F. Serum complement in acute glomerulonephritis and the nephrotic syndrome. *Pediatrics*, 1951, **8**, 814–820.

31. Schwab, L.; Moll, F. C.; Hall, T.; Brean, H.; Kirk, M.; Hawn, C. V. Z., and Janeway, C. A. Experimental hypersensitivity in the rabbit. Effect of inhibition of antibody formation by x-radiation and nitrogen mustards on the histologic and serologic sequences, and on the behavior of serum complement, following single large injections of foreign proteins. *J. Exper. Med.*, 1950, **91**, 505–526.

32. Mellors, R. C., and Ortega, L. G. In preparation.

[*Illustrations follow*]

LEGENDS FOR FIGURES

Figs. 1 to 17. Perfused rabbit organs. Sections stained with hematoxylin and eosin unless otherwise specified.

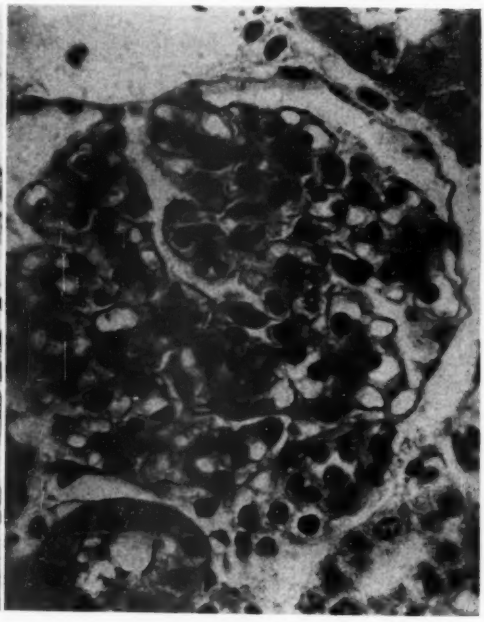
FIG. 1. Control rabbit. Normal glomerulus displaying thin-walled patent capillaries. × 475.

FIG. 2. Rabbit B. Glomerulitis with swelling of cells, widening of basement membrane, and reduction of the lumen of the capillaries in the glomerulus. × 475.

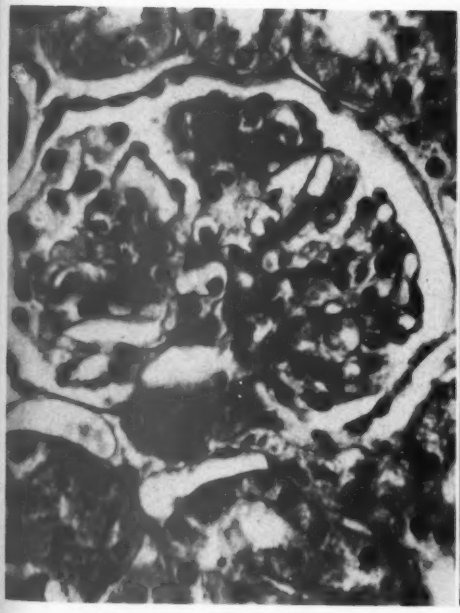
FIG. 3. Rabbit Q. Glomerulitis with deposition of large masses of PAS-staining granular material in the glomerulus. Periodic acid-Schiff (PAS) stain. × 475.

FIG. 4. Rabbit Q. Glomerulitis with thickening of the basement membrane and widening of the intercapillary spaces, the change being more prominent in one half of the glomerulus. × 475.

1

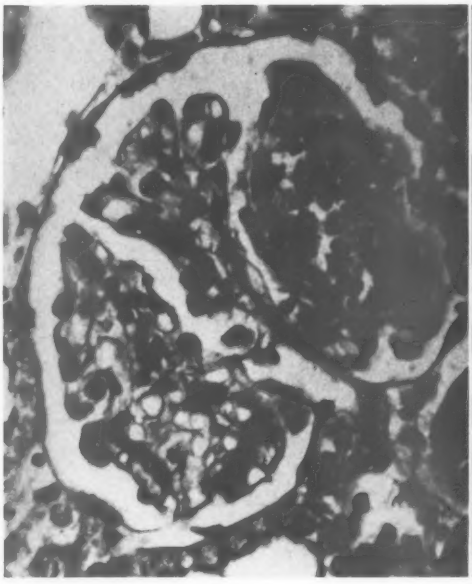


2

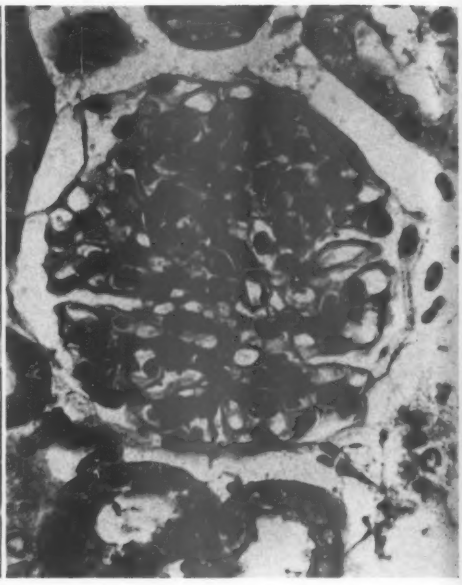


3

4



5



6

FIG. 5. Rabbit Q. Focal proliferative glomerulitis. PAS stain. × 475.

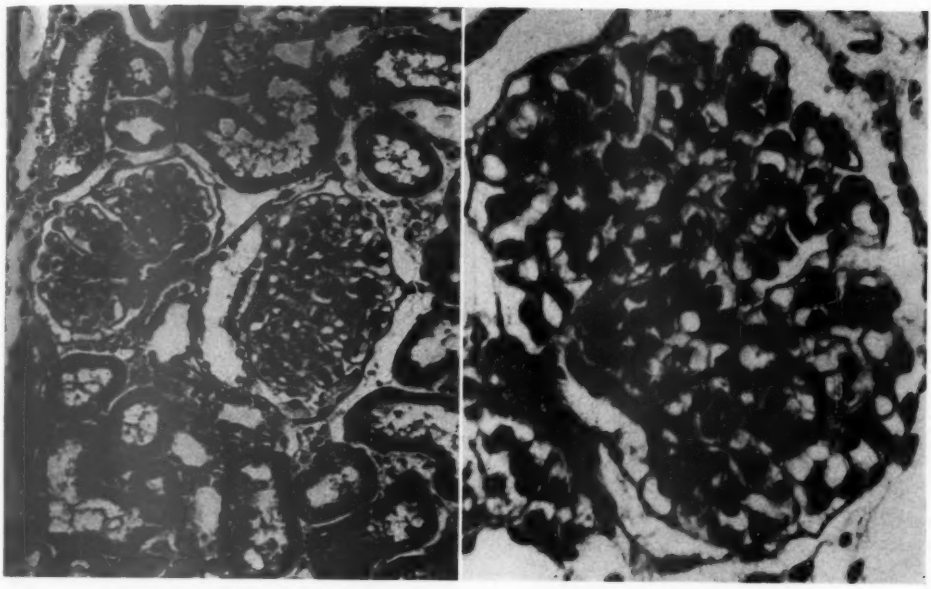
FIG. 6. Rabbit G. Proliferative glomerulitis, more accentuated in one half of the glomerulus. × 475.

FIG. 7. Rabbit B. Diffuse proliferative glomerulitis of moderate severity accompanied by alterations in the capillaries. × 195.

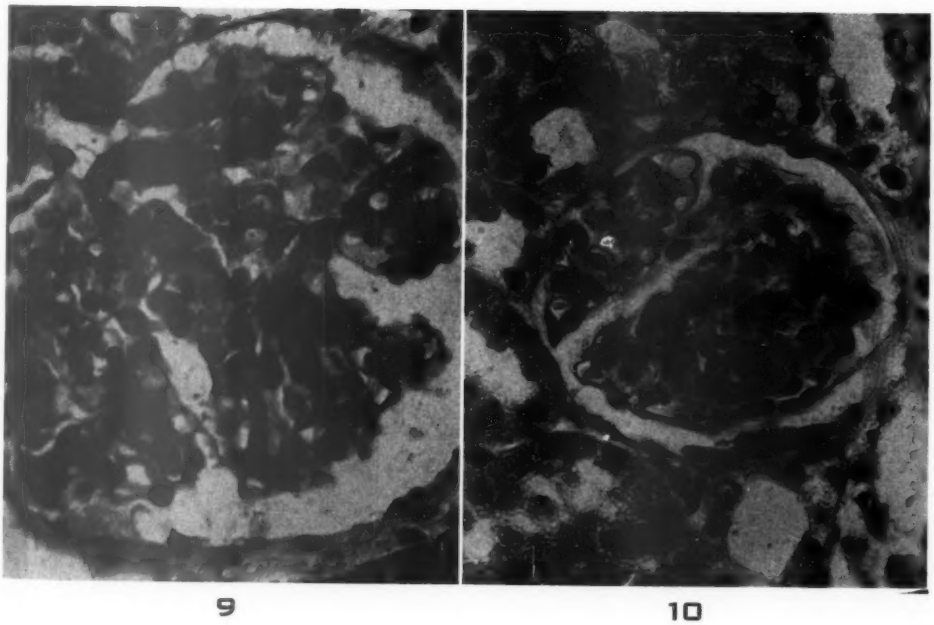
FIG. 8. Rabbit Q. Diffuse proliferative glomerulitis with uniform involvement of the glomerulus. × 470.

FIG. 9. Rabbit E. Diffuse exudative and proliferative glomerulitis. × 470.

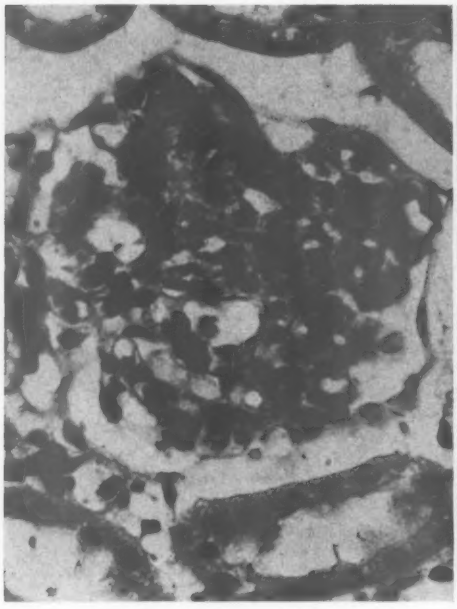
FIG. 10. Rabbit Q. Glomerulitis with focal area of early necrosis in which nuclear fragmentation is conspicuous. × 470.



7 8



9 10



11

12

FIG. 11. Rabbit G. Proliferative glomerulitis with early fibrosis and retraction. × 480.

FIG. 12. Rabbit F. Proliferative glomerulitis with more advanced intracapillary and extraglomerular fibrosis. × 480.

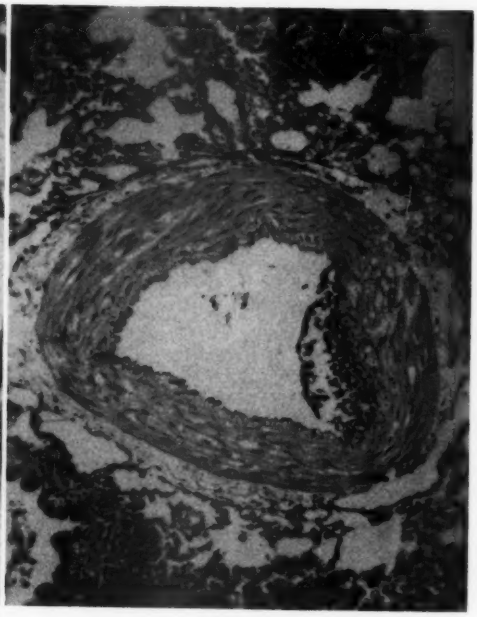
FIG. 13. Rabbit R. Glomerular crescent with adhesion between the capillary tuft and Bowman's capsule. × 480.

FIG. 14. Rabbit G. Endarteritis in a small pulmonary artery. × 95.

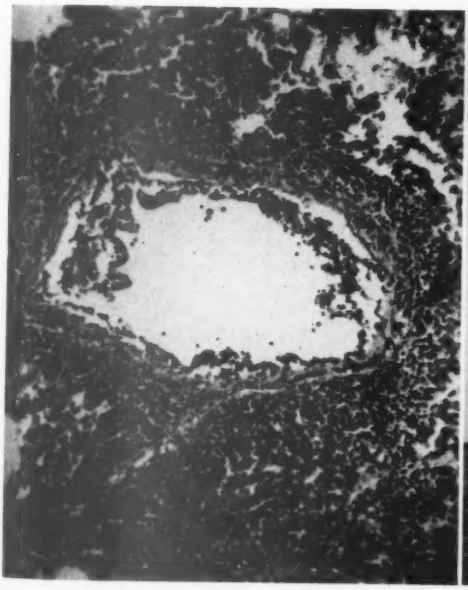
FIG. 15. Rabbit G. Panvasculitis and interstitial pneumonitis. × 95.

FIG. 16. Rabbit C. Mitral valve with acute valvulitis and a large focus of fibrinoid necrosis. × 95.

13



14



15

16

17

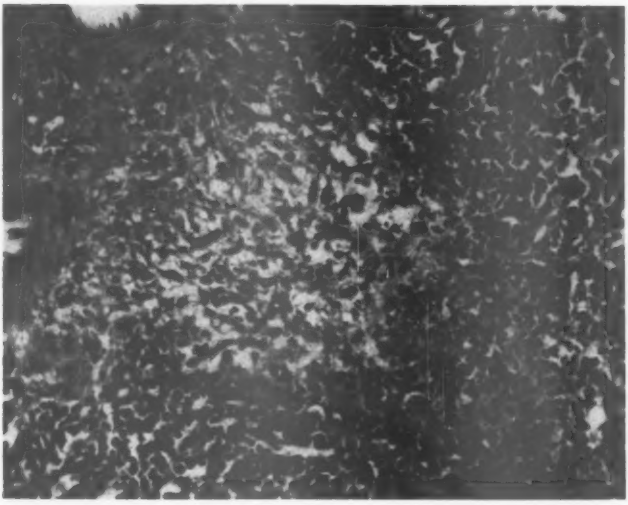
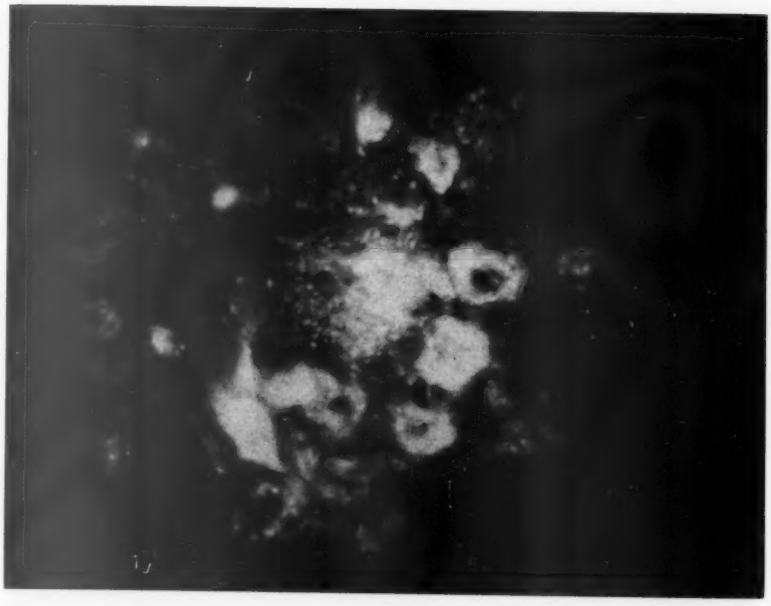


FIG. 17. Rabbit G. Spleen with perivascular allergic granuloma. × 175.

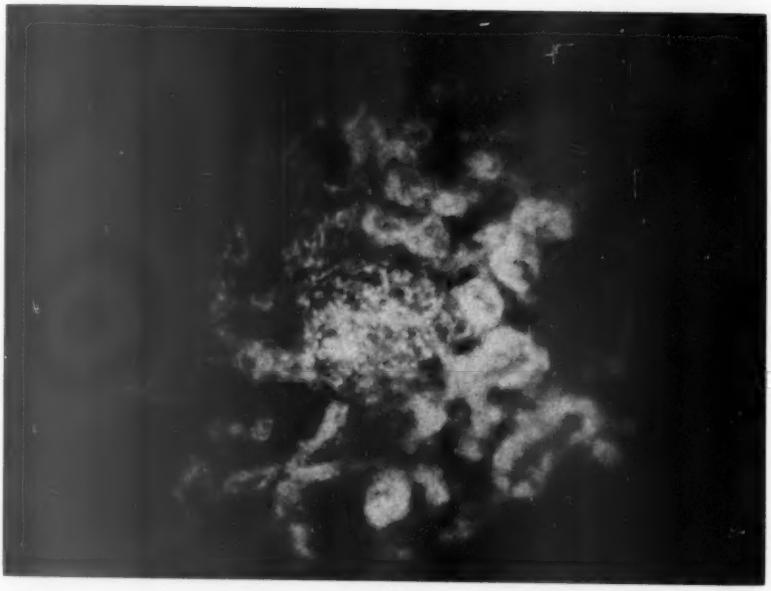
Figs. 18 to 30. Rabbit kidneys. Unfixed, frozen sections treated with the fluor (fluorescein-labelled Chi-anti-Rab) to demonstrate the localization of antibodies by fluorescence. Fluorescence photomicrographs except where otherwise stated. × 200.

FIG. 18. Normal control rabbit. Normal glomerulus and tubules. The fluorescence in the section is due principally to non-specific combination with the fluorochrome. The fluorescence intensity of the glomerulus is slightly greater than that of many of the tubules.

FIG. 19. Another normal control rabbit. Normal glomerulus and tubules.



18

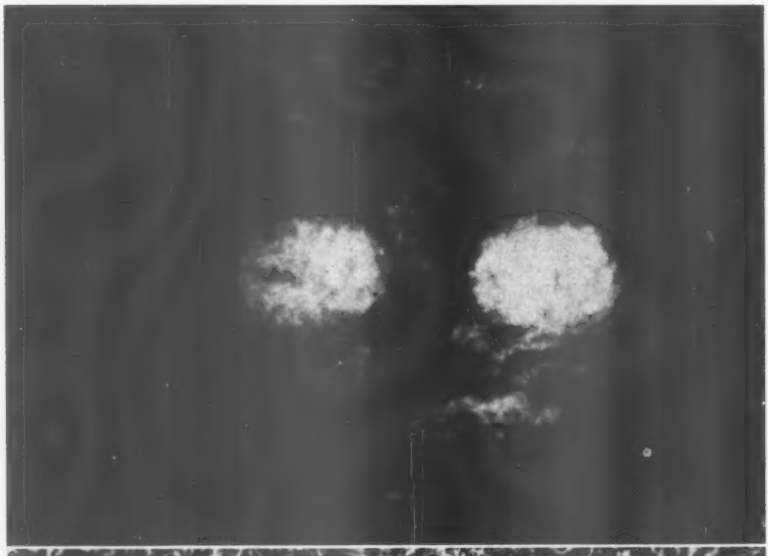


19

FIG. 20. Kidney of a rabbit (Q) having proliferative glomerulitis. The fluorescence intensity of the glomeruli is much greater than that of the tubules.

FIG. 21. Phase photomicrograph of the same field seen in Figure 20. × 200.

20



21

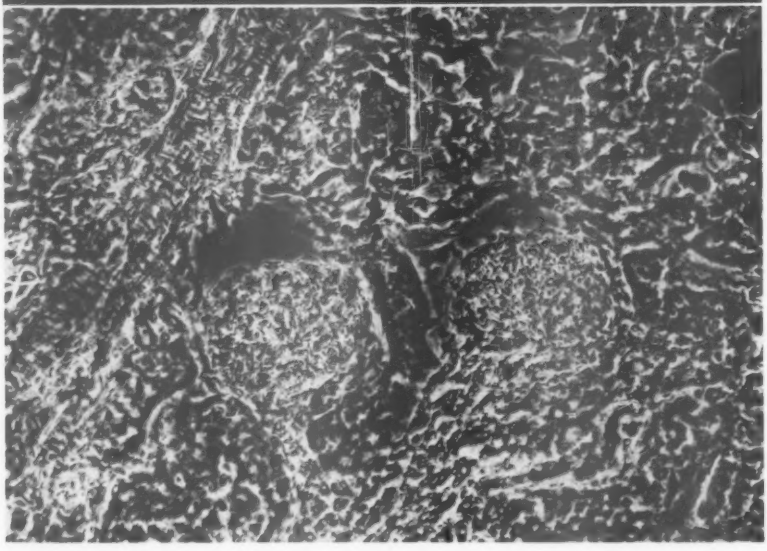
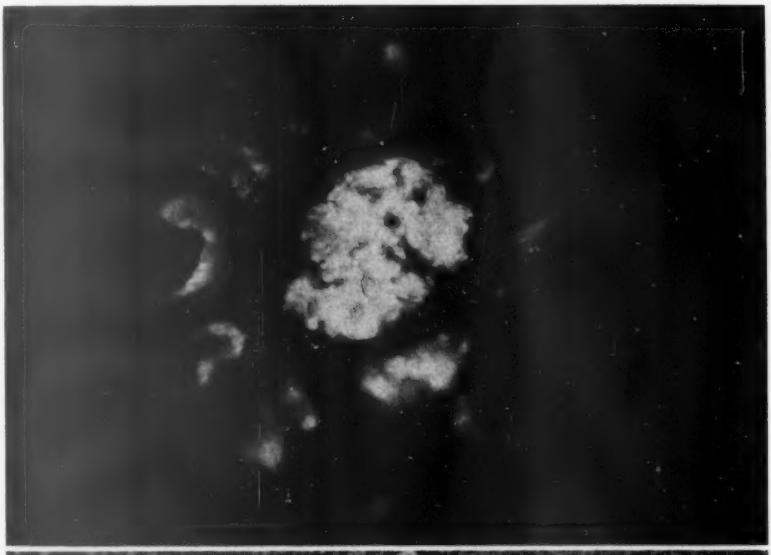
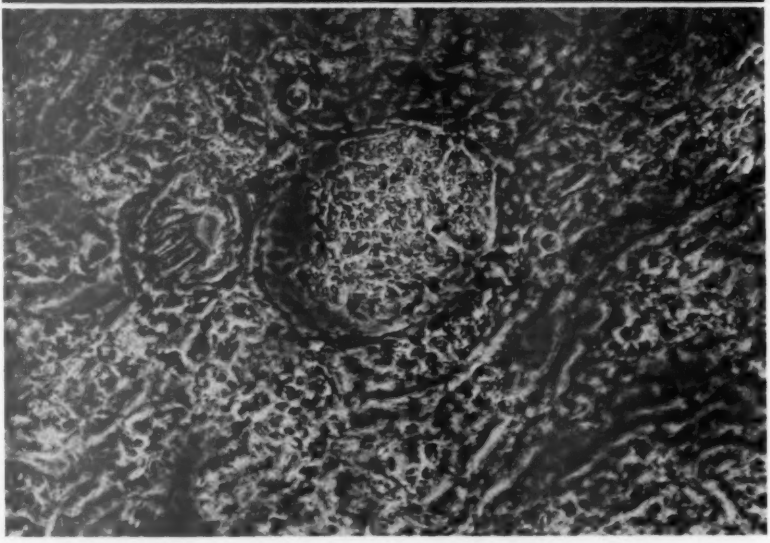


FIG. 22. Kidney of a rabbit (R) having proliferative glomerulitis. The fluorescence intensity of the glomerulus is much greater than that of tubules.

FIG. 23. Phase photomicrograph of same field seen in Figure 22. × 200.



22



23

FIG. 24. Kidney of a rabbit (R) having proliferative glomerulitis. The specific fluorescence has been blocked, however, by treatment of the section with unlabelled globulin (Chi-anti-Rab) prior to using the fluor. The fluorescence intensity of the glomerulus is then only slightly greater than that of the tubules, indicating that much of the glomerular fluorescence illustrated in Figures 22 and 23 is inhibited by this immunochemical procedure. The fluorescence which can be specifically blocked in this manner is therefore due to the presence of localizing antibodies which are concentrated in the glomerulus.

FIG. 25. Another microscopic field with conditions identical to those in Figure 24.

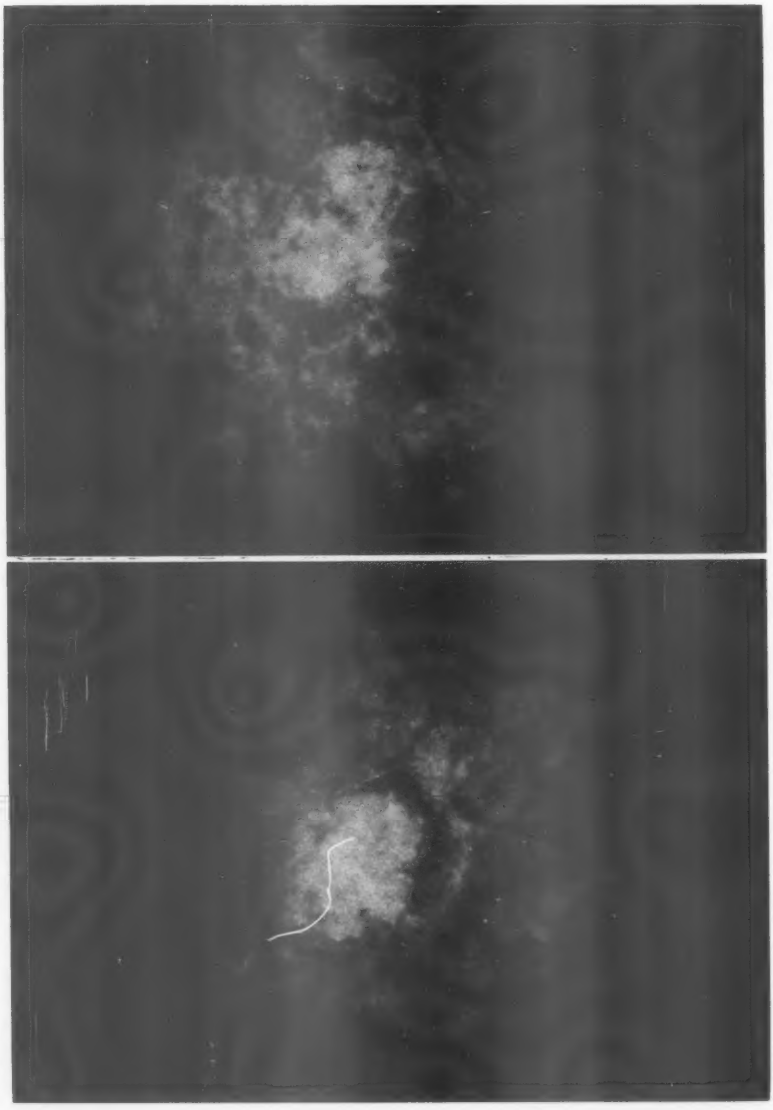
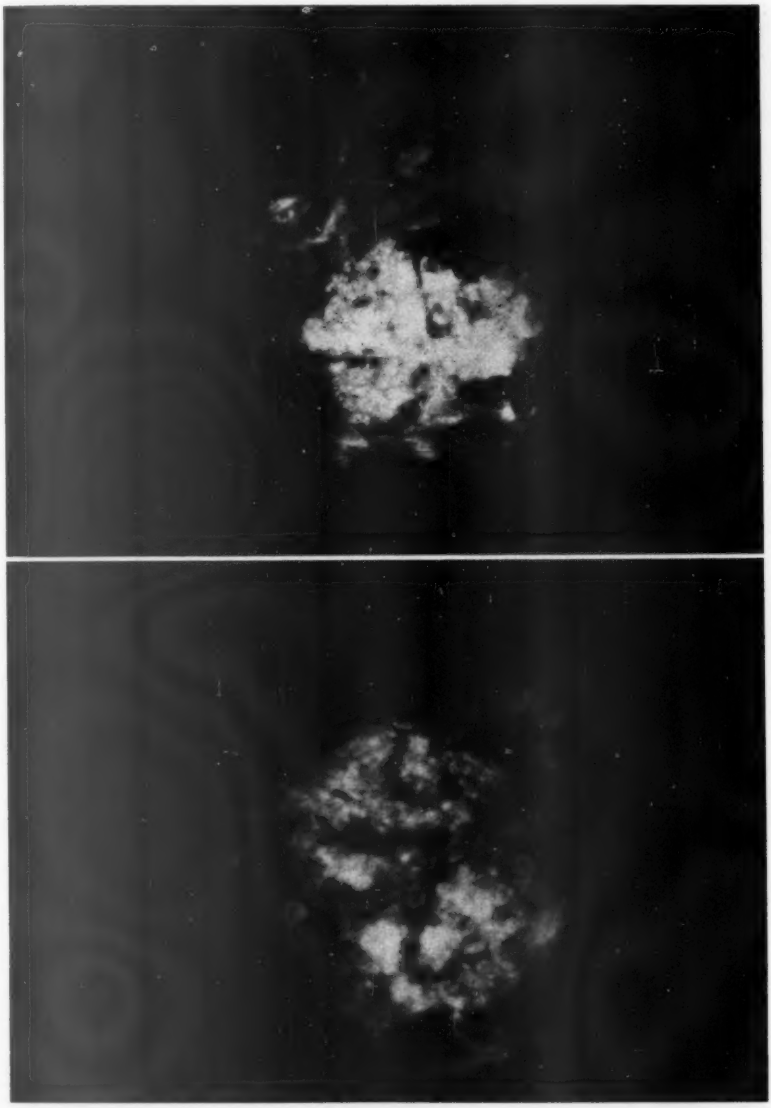


FIG. 26. Kidney of a rabbit (N) having glomerulitis and crescent formation. The relative fluorescence intensity of the glomerulus is increased.

FIG. 27. Kidney of a rabbit (E) having proliferative and exudative glomerulitis. The relative fluorescence intensity of the glomeruli is increased.



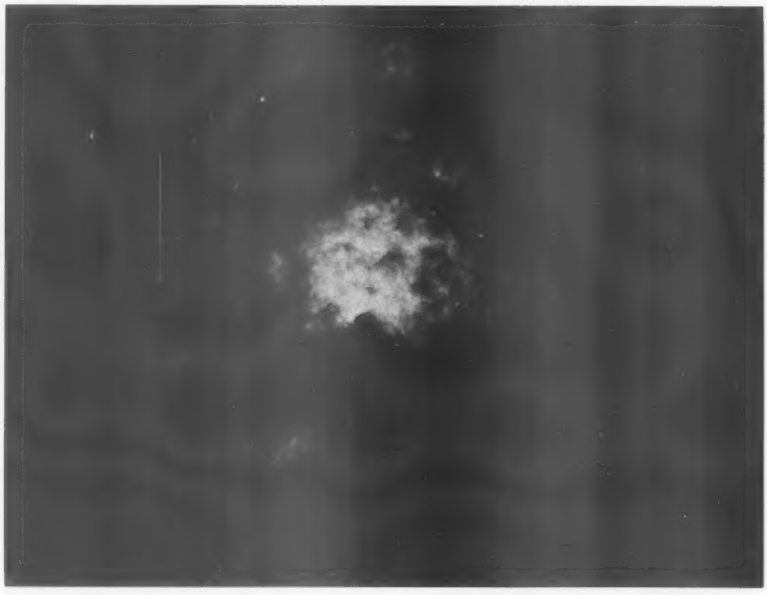
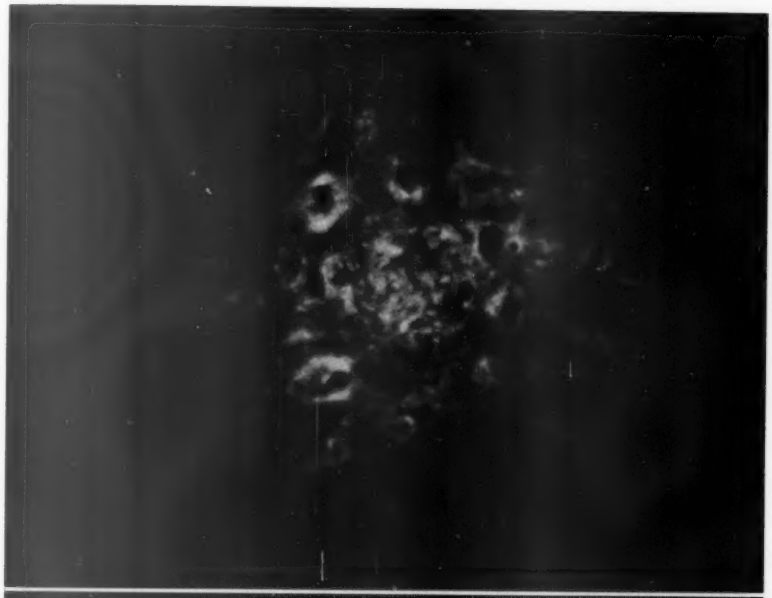


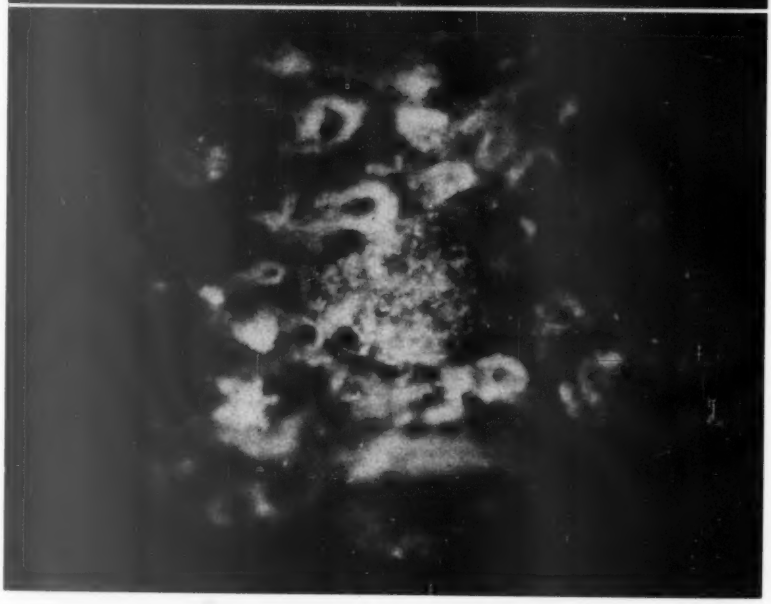
FIG. 28. Kidney of a rabbit (G) having proliferative glomerulitis. The relative fluorescence intensity of the glomerulus is increased.

FIG. 29. Kidney of a rabbit (C) having glomerulitis with capillary change only. The fluorescence intensity of the glomerulus is only slightly greater than that of the tubules.

FIG. 30. Kidney of a rabbit (T) which received injections of bovine gamma globulins but did not develop glomerulitis. The fluorescence intensity of the glomerulus is only slightly greater than that of the tubules.



29



30

EXPERIMENTAL ARTERIOPATHY

Spontaneous, Epinephine-Thyroxine, and Cholesterol-Induced Forms *

Y. T. Oester, M.D.; Oscar F. Davis, Ph.D., and Bernard Friedman, B.S.
From the Department of Pharmacology, Stritch School of Medicine (Dr. Oester, Dr. Davis, and Mr. Friedman) and the Graduate School of Loyola University (Mr. Friedman), Chicago, Ill.

This study was undertaken in order to establish methods for the production of experimental arteriopathy which would lend themselves to a broad evaluation of factors influencing arteriosclerosis. In addition, it was thought that such studies might provide a better understanding of the pathogenesis of the lesions. In this program, two sclerogenic regimens were employed. One of these, employing cholesterol, produced lesions not unlike intimal atherosclerosis of humans. The other, using epinephrine-thyroxine, induced a degeneration, essentially similar to medial arteriosclerosis or arterionecrosis.

Josué[1] and others have demonstrated the sclerogenic effects of repeated intravenous injections of epinephrine.[2] Davis and Oester[3] observed a greater incidence and a more severe medial necrosis when thyroxine was added to the regimen of epinephrine injections. The arteriopathy that supervened was medial and usually non-atheromatous. In contrast, when cholesterol alone was utilized to induce experimental sclerosis, the lesions that resulted were intimal and atheromatous. Another part of this investigation dealt with aortic sclerosis induced when cholesterol and epinephrine-thyroxine programs were administered jointly to the same animal.

The literature contains descriptions of the spontaneous development of microscopic atherosclerosis in rabbits.[4] Such reports have been accepted by some as indicative of an almost universal spontaneous atherogenic pattern in these animals. Spontaneous macroscopic lesions, histologically equivalent to those induced by epinephrine, have been found in 15 per cent of 210 rabbits.[5] Similar spontaneous medial lesions were observed by Miles.[6] On the other hand, Hedinger,[7] in a study of 100 rabbits, found no evidence of arterial disease.

In order to assess spontaneous arterial sclerogenesis as a factor in the present study, 84 rabbits, not on any known sclerogenic schedule, were examined for arteriopathy.

* This investigation was supported by grants from the National Heart Institute, National Institutes of Health, Public Health Service, and from the Chicago Pharmacal Company, Chicago, Ill.

Received for publication, October 26, 1954.

Methods

For this work a total of 210 adult albino rabbits, of both sexes, were used. They weighed from 1.5 to 3.5 kg.

Epinephrine-Thyroxine Sclerosis

Sixty animals were treated as follows. On 2 consecutive days, 25 μg. of epinephrine hydrochloride U.S.P. per kg. of body weight were injected very slowly by ear vein. On the third and fourth days the dose of epinephrine was increased to 40 μg. per kg. On the fifth day, it was increased to 50 μg. per kg. This regimen of 50 μg. of epinephrine per kg. of body weight was maintained for 11 more days, making a total of 15 days of such injections. An aqueous preparation of thyroxine was administered subcutaneously in the region of the middle of the back, daily, for each of these 15 days. This was at a constant dose level of 0.15 mg. per kg. of body weight per day.

Cholesterol Sclerosis

Slow, intravenous administrations of cholesterol suspensions were carried out in 43 animals on a daily dosage schedule of 40 mg. of cholesterol per kg. of body weight. This amount was divided into two injections, each of approximately 20 mg. per kg., separated by at least 2 hours. These intravenous injections were administered for a total of 20 days. In addition, 600 mg. per day of cholesterol, in two separated doses of 300 mg. each, were administered subcutaneously from the 10th to the 20th day (11 days).

The cholesterol, 25 mg. per ml., was suspended in distilled water by use of 9 mg. of sodium chloride, 9 mg. of benzyl alcohol, 5 mg. of sodium carboxymethylcellulose, and 4 mg. of Tween 80.

The vehicle alone, each ml. of which contained the sodium chloride, benzyl alcohol, sodium carboxymethylcellulose, and Tween 80 in exactly the same concentration as in the cholesterol suspension, but contained no cholesterol, was injected into a third group of 12 rabbits on exactly the same schedule and in the same amount as that with cholesterol.

Combined Sclerosis

Eleven other animals were treated with combined injections of epinephrine-thyroxine and cholesterol under the same conditions as have been described for each separately.

The process of inducing relatively acute aortic lesions in 15 to 20 days is a debilitating procedure to the animals involved, and results

in a significant number of early deaths. For this reason, all animals failing to survive to the sixth day of injections were eliminated from the final analysis.

Spontaneous Sclerosis

Eighty-four rabbits, not on any known sclerogenic regimen, were examined for evidence of lesions of the aorta.

General Procedures

Post-mortem examinations were conducted routinely on each animal in this study. Our primary observations were concerned with the frequency and severity of macroscopic arteriopathy in the aorta from its origin to the iliac bifurcation. The ineffectiveness of macroscopic grading of such sclerotic changes leads to difficulty in assessing the extent of these lesions in any comparative sense. However, for purposes of differentiation, we have undertaken grading them on an arbitrary scale of increasing severity from zero to four: 0, no sclerotic patches; 1, single sclerotic patch; 2, several sclerotic patches; 3, scattered extensive sclerotic patches; 4, widespread confluent and extensive sclerotic patches.

In addition to this numerical method for recording the degree of sclerosis, we have employed three other criteria in estimating the extent of arteriopathy. These are: (a) percentage incidence, the ratio of animals with sclerosis divided by the total number of animals in a group; (b) per cent of animals showing gross arteriopathy of degree 3 or 4; (c) severity factor, a number derived from the multiplication of the percentage incidence by the average degree of sclerosis (0 through 4).

Microscopic studies of selected specimens of aortas were made, utilizing routine hematoxylin and eosin staining methods. In addition, some were examined for sudanophilia.

Results

The entire group of 84 basic control animals was examined for spontaneous development of gross arteriopathy. Sixty-one had received no injections. Fourteen, comprising a second portion of this control group, were obtained from the student laboratory of pharmacology. They had been subjected to a single injection of sodium salicylate. A third portion of the basic controls consisted of 9 rabbits which had been exposed to one injection of 1 ml. of horse serum. In this entire group of 84, only one animal (from the horse serum group) showed

TABLE I

Evaluation and Comparison of Sclerogenic Regimens

Treatment	Total number of rabbits	Early deaths	Number with sclerosis over total number of surviving animals	Incidence	Degree					Average degree	Animals reaching degree 3 or 4	Severity factor (per cent of incidence × average degree)
					0	1	2	3	4			
				%							%	
Epinephrine-thyroxine	60	12	43/48*	89.5	5	4	10	13	16	2.6	60.4	233
Cholesterol suspension	43	11	23/32	71.8	9	2	8	4	8	1.9	37.5	136
Epinephrine-thyroxine plus cholesterol	11	5	6/6	100	0	0	0	1	5	3.8	100	380
Vehicle minus cholesterol	12	0	0/12	0	12	0	0	0	0	0.0	0	0.0
No sclerogenic treatment	84	0	1/84	1.1	83	1	0	0	0	0.01	0	0.01

* Data includes 20 animals from a previous study.[3]

signs of arteriopathy. Sixty rabbits, which included 20 used for a previous report,[3] were used in one experimental group and were treated with epinephrine and thyroxine. Forty-eight survived until after the sixth day of injections (12 early deaths). Of these 48 animals, 43 showed grossly visible signs of arteriopathy. Histologic examination of selected specimens showed these lesions to be of an essentially non-atheromatous, medial type. Sometimes intimal involvement was observed also. Very often, the extent of these changes was severe. In many local areas almost complete disappearance of the tunica media was seen.

A second group, 43 animals, was subjected to injections of the cholesterol suspension. Thirty-two survived until after the sixth day (11 early deaths). Of these 32, 23 showed macroscopic lesions of the aorta. Microscopically, these lesions were intimal and atheromatous, and were relatively less extensive than those induced by epinephrine-thyroxine. This was in contrast to the 12 animals which were exposed to injections with the cholesterol-free aqueous vehicle used with cholesterol. None of these animals exhibited evidence of sclerosis.

To another group of 11 animals, epinephrine-thyroxine

and cholesterol injections both were administered, in the same manner as when given separately. Of the original group, only 6 survived after the sixth day (5 early deaths). Each of them had, grossly, a severe sclerosis. Histologically this appeared to be a combined form of arteriopathy. The intimal proliferation appeared, as a rule, to be much more extensive than when cholesterol alone was used.

Discussion and Conclusion

The incidence of spontaneous aortic sclerogenesis in our rabbit population was determined in 84 rabbits not exposed to any known sclerogenic schedule. The presence of macroscopic lesions in only one of these 84 rabbits leads to the conclusion that spontaneous gross sclerogenesis was not a significant factor in this study.

In this analysis, particular emphasis was placed on the incidence of gross evidence of sclerosis, not, however, to the exclusion of microscopic studies. This was based on the practical reason that clinical arterial disease of any type invokes its gravest influence only as it tends to become occlusive. Thus, even long-standing and extensive medial necrosis is less likely to have a lethal effect than atherosclerosis which, though less extensive and severe, often is occlusive.

Our own studies and theories, added to the recent reports of others (American Society for the Study of Arteriosclerosis,[8] Crawford and Levene[9]), suggest that intimal atherosclerosis, and medial arteriosclerosis and arterionecrosis are intimately interrelated. Our experimental and theoretic separation of the more dangerous component, atherosclerosis, from the essentially medial form does not lessen our interest in either phase. This work has resulted in the production of an essentially medial necrosis, not dissimilar from human medial arteriosclerosis or arterionecrosis. An obvious advantage of this method of induction is in the unusually high incidence (89.5 per cent) of lesions and the rapidity with which they developed (6 to 15 days). These lesions only infrequently showed a proliferating intima. The damage, however, was at times destructive of large sections of the media. The basis for these effects of epinephrine may not reside alone in its hypertensive or ischemia-producing properties, but may also be related to interference with indispensable metabolic pathways in the utilization and distribution of lipid, protein, and carbohydrate.

The potentiated arteriopathy in animals receiving thyroxine as well as epinephrine (Davis and Oester[3]) may possibly be based on thyroxine-induced sensitization to sympathetic discharges, epinephrine, or arterenol activity. The rôle of thyroxine in such instances is somewhat

paradoxical. Even as it augments the development of medial lesions, in association with epinephrine, it is said to inhibit, or at least retard, the development of cholesterol-induced atheromatosis.[10] Furthermore, evidence has been presented by Moses and Longabaugh[11] and Firstbrook[12] that weight gain or weight maintenance is a necessary factor in atherogenesis. Conversely, weight loss is said to favor a degree of inhibition of cholesterol-induced atherosclerosis. Epinephrine-thyroxine animals (medial arteriopathy) and epinephrine-thyroxine-cholesterol animals (combined arteriopathy) all lose weight drastically, and yet develop severe sclerosis in the presence of a high dosage of thyroxine. The rôle of thyroxine or of the thyroid gland in epinephrine-thyroxine sclerosis is apparently diametrically opposite to the influence of thyroxine in cholesterol-induced arteriopathy.

Lesions, primarily of the intima, and of an atheromatous type, were produced by the intravenous injection of saline suspensions of cholesterol. The morphologic changes in the aortas of animals under this regimen are similar to the atherosclerotic changes in humans, as well as to those produced by experimental oral cholesterol feeding.

When cholesterol and epinephrine-thyroxine regimens were combined, an arteriopathy resulted which was clearly an exaggeration of the type induced separately by each. It was both medial and intimal. In the small group of animals surviving this regimen the intimal proliferation and degree of foam cell formation appeared, as a rule, greater than when cholesterol alone was injected, and was of an order more closely resembling prolonged experimental cholesterol feeding.

These experimental studies in combined arteriopathy stem from the earlier postulate of Davis and Oester[8] that the pathogenesis of atherosclerosis does not reside alone, or even primarily, in excesses or metabolic errors of cholesterol. Neither does it necessarily reside in the intimal layers alone. Influences involving the intermediary metabolism of the artery as a whole, perhaps, specifically, the media, may be involved in a primary way. Imbalances in epinephrine or epinephrine-like factors may be *one* of the factors which will provoke the metabolic, as well as morphologic, derangement necessary to initiate sclerosis and, under certain conditions, atherosclerosis.

An interrelationship between atherosclerosis and medial arteriosclerosis is *now* widely accepted. The Nomenclature Committee of the American Society for the Study of Arteriosclerosis[8] stated that the combination of atherosclerosis and medial arteriosclerosis or arterionecrosis is frequent. It was suggested also that medial lesions may often determine the localization of atheroma in susceptible individuals.

Bell[13] stated that intimal atherosclerosis is so frequently superimposed on medial calcification that differentiation is difficult. Recent striking evidence of a nearly universal tendency to thinning of the media in 134 aortas from human cases of atherosclerosis presumably uncomplicated by any medial degeneration, as reported by Crawford and Levene,[9] adds credence to these suggested relationships.

From our limited survey, one published case history emphasized the existence of widespread atherosclerosis in a young patient with pheochromocytoma (Kremer[14]). This process was of an order not to be expected in the younger group, and provides some added support for these relationships. This should be compared to the effects that may occur under the influence of epinephrine or epinephrine-like compounds.

In addition, the failure to find cholesterol in the earliest lesions of intimal atherosclerosis (Klatzo, McMillan, and Duff[15]) and the not uncommon existence in young people of non-atheromatous arteriopathy (Hueper[16]) are further indications that cholesterol excess or errors of metabolism of cholesterol may not be the only or even the primary factor in the production of atherosclerosis.

Summary

Examinations of aortas for gross arteriopathy, from rabbits on no experimental sclerogenic program, demonstrated only one lesion in 84 animals.

Rabbits receiving epinephrine intravenously and thyroxine subcutaneously exhibited an essentially medial arteriosclerosis. This was of higher incidence and more severe than that produced by epinephrine alone.

Intravenous injection of cholesterol suspensions resulted in a high incidence and rapid onset of a proliferative, intimal atherosclerosis.

Exposure to simultaneous epinephrine-thyroxine and cholesterol regimens resulted in a combined arteriopathy which demonstrated a far greater severity of arteriosclerosis (atheromatosis in particular) than from either program alone.

We wish to express our sincere appreciation to Merck & Co., Inc., Rahway, N.J., for cholesterol, which they generously supplied for this study.

REFERENCES

1. Josué, O. Athérome aortique expérimental par injections répétées d'adrénaline dans les veines. *Presse méd.*, 1903, 11, 798–799.
2. Mikulicich, G., and Oester, Y. T. Hormonal influences on epinephrine induced aortic sclerosis. *Proc. Soc. Exper. Biol. & Med.*, 1951, 78, 816–818.

3. Davis, O., and Oester, Y. T. Experimental arteriosclerosis: inhibitory effects of ascorbic acid and inositol. *Proc. Soc. Exper. Biol. & Med.*, 1952, 81, 284–286.
4. Bragdon, J. H. Spontaneous atherosclerosis in the rabbit. *Circulation*, 1952, 5, 641–646.
5. Hill, M. C. Various forms of experimental arterial disease in the rabbit. *Arch. Int. Med.*, 1910, 5, 22–29.
6. Miles, A. B. Spontaneous arterial degeneration in rabbits. *J. A. M. A.*, 1907, 49, 1173–1176.
7. Hedinger, E. Ueber experimentell durch Adrenalin und Hämostasin erzeugte Arterienerkrankungen bei Kaninchen. *Cor.-Bl. f. schweiz. Aerzte*, 1905, 35, 634–645.
8. Annual Report, Nomenclature Committee, American Society for the Study of Arteriosclerosis. Special Bulletin, 1953, 1, 1–5.
9. Crawford, T., and Levene, C. I. Medial thinning in atheroma. *J. Path. & Bact.*, 1953, 66, 19–23.
10. Katz, L. N. Experimental atherosclerosis. *Circulation*, 1952, 5, 101–114.
11. Moses, C., and Longabaugh, G. M. Evaluation of choline in the prevention of experimental atherosclerosis: importance of changes in body weight. *Arch. Path.*, 1950, 50, 179–182.
12. Firstbrook, J. B. The effect of changes in body weight on atherosclerosis in the rabbit. *Science*, 1950, 111, 31–33.
13. Bell, E. T. A Text-book of Pathology. Lea & Febiger, Philadelphia, 1952, ed. 7, pp. 363–364.
14. Kremer, D. N. Medullary tumor of the adrenal glands with hypertension and juvenile arteriosclerosis. *Arch. Int. Med.*, 1936, 57, 999–1007.
15. Klatzo, I.; McMillan, G. C., and Duff, G. L. Histochemical studies of the early lesions of cholesterol atherosclerosis in the rabbit. *Circulation*, 1952, 6, 464–465.
16. Hueper, W. Arteriosclerosis. *Arch. Path.*, 1944, 38, 162–181, 245–285, 350–364.

MITOCHONDRIAL CHANGES IN HEPATIC CELLS OF X-IRRADIATED MICE *

ROSS C. MACCARDLE, Ph.D., and CHARLES C. CONGDON, M.D.†
From the Laboratory of Pathology and the Radiation Branch, National Cancer Institute, Bethesda, Md.

Wilhelm Roentgen published in 1895 his celebrated discovery of the x-ray for which he was awarded the first Nobel Prize in Physics in 1901. More than 100 years ago, Virchow, in 1854, wrote of myelin forms in pathologic tissue, later to be identified as phospholipids of the cell. Mitochondria were brought into prominence by the brilliant researches of Richard Altmann,[1] in 1890, when he described his *granula*, earlier known as *cytomicrosomes* by Valette St. George[2] and *fila* by Flemming.[3] Most of Altmann's granules and the organelles now termed mitochondria are indisputably identical. Altmann considered them to be elementary organisms (*Bioblasten*) in the cell. Fischer[4] declared that Altmann's granules were artifacts of fixation. Verworn[5] vigorously attacked Altmann's hypothesis because none of the granules possessed vital phenomena. Bütschli[6] had shown that free-living bacteria are complete cells unlike Altmann's granules. Nonetheless, the importance of the granules in pathology was long ago recognized as seen by the early literature of the Ergebnisse of Lubarsch and Ostertag (also Galeotti[7]). On July 29, 1898, the pathologist Benda[8] introduced the term mitochondria to characterize some of Altmann's granules. The investigations of Michaelis,[9] in 1900, were paramount, for he found the vital dye Janus green (diethylsafraninazodimethylanilin) to be specific in coloring certain cytoplasmic filaments. This led first Fauré-Fremiet,[10] then Bensley,[11] Cowdry,[12] and the Lewises[13] to prove that mitochondria exist in living protoplasm.

It is now 60 years since these great events, and there is still no comprehensive account of the effect of x-irradiation on the mitochondria *in situ* in *normal* cells of animal tissue. Moreover, little is known of the action of x-rays on phospholipids, an important chemical constituent of the mitochondrion. This would seem to be a logical point of attack on the problem of x-irradiation injury in view of the fact that lecithin on hydrolytic decomposition yields the ptomaine choline (Mathews[14]).

Kingsbury[15] believed the mitochondria to be autoxidizable phospholipid-protein complexes that serve in cellular respiration. Fauré-

* Received for publication, December 3, 1954.
† Now at the Division of Biology, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Fremiet[16] extracted from the ovary phospholipids that resembled mitochondria. Mayer, Rathery, and Schaeffer,[17] in their famous discovery of the *élément constant* of phospholipids, showed that when mitochondria were increased in the liver, the phospholipids of the same liver also increased. In their experimental production of *pâté de foie gras* in geese, they found that as mitochondria decreased, so too the phospholipids decreased. Löwschin[18] made artificial myelin forms from lecithin in albumin solutions and thus formed granules, rods, and filaments of lecithalbumin that could be fixed and colored like mitochondria. Bensley and Hoerr[19] succeeded in separating from the liver mitochondria that consisted chiefly of phospholipids and protein.

Kögel[20] reported that lecithin (of egg yolk) was decomposed upon exposure to x-rays. Potter and Bethell[21] isolated mitochondria from the spleen of normal rats, and then x-irradiated the mitochondria in a medium of sucrose. They found no effect on the phosphorylating capacity of the mitochondria thus irradiated in sucrose. However, they found this function to be reduced markedly in mitochondria isolated from previously irradiated rats. Beckton and Colwell[22] believed x-rays to have no effect on "Altmann's granules" (?) in slices of spleen x-irradiated after excision from normal mice, whereas Beckton and Russ[23] said that radium induced dissolution of the granules. Entenman and Weinman[24] found that slices of liver of x-irradiated rats incorporated more inorganic phosphorus into their phospholipids than those of normal animals as early as 14 hours after exposure, and the liver was heavier after x-irradiation.

Gatenby and his associates[25] found that mitochondria of sperm-forming cells were vesiculated and finely granular following exposure to x-rays. Hirsch[26] described fragmenting of mitochondria in acinar cells of the pancreas after x-irradiation. Bloom[27] stated that the mitochondria of the cells of uriniferous tubules of the kidney of x-irradiated rats (600 r.) showed no change whatsoever, and the mitochondrial changes in the more "radiosensitive" intestinal epithelium did not occur until after the appearance of degenerative nuclear damage. Bloom[28] expressed the opinion that irradiation induces damage in the nucleus prior to morphologically detectable cytoplasmic damage. Tschassownikow[29] clearly demonstrated that in x-irradiated summer and winter frogs the mitochondria of the liver were globulated* and

* *Globulated, globulation,* and *globulating* have not been accepted by the dictionaries which establish standards of spelling for *The American Journal of Pathology*. The meanings of these words are self-evident and they are used throughout this article in order to avoid cumbersome circumlocution.—*Editor.*

fragmented, and he believed that this occurred prior to injury of the nucleus.

Heilbrunn and Mazia[30] pointed out that x-irradiation altered the cytoplasm of the sea urchin egg by liberating calcium into the cell. Cleavage of the cytoplasm was markedly delayed in division of fertilized sea urchin eggs exposed to x-rays, and the delay was prolonged in the presence of x-irradiated ovarian tissue (Heilbrunn and Young[31]). Congdon, Uphoff, and Lorenz[32] found that injection of bone marrow of a non-irradiated guinea-pig into x-irradiated animals stimulated regeneration of injured bone marrow but did not prevent its initial destruction by x-rays. Duryee[33] found that isolated nuclei of immature eggs of the frog were relatively insensitive to x-rays, but when he injected irradiated cytoplasm into normal eggs, the nuclei of the non-irradiated eggs were affected. The excellent experiments of Wilson[34] demonstrated that x-irradiation retarded the cytoplasmic liquefaction that normally follows gelation prior to mitotic division of sea urchin eggs, whereas after a period of recovery the eggs proceeded to apparently normal development. In respect to the locus of early cellular damage, it should be remarked that Policard[35] showed that heat altered the mitochondria of renal cells without visible damage to the nucleus. One of us (R.C.M.)[36,37] has demonstrated marked globulation and vesiculation of mitochondria in the liver after subjection of poikilothermic animals to nearly lethal high temperatures from which they could recover.

The present paper deals chiefly with the comparison of early changes in the form and distribution of mitochondria in the hepatic cells of mice following exposure of the entire body to 500, 900, or 1,200 r. of irradiation. No analysis of the phospholipids of the liver was undertaken. The effect of intravenously injected bone marrow on the mitochondria was of primary interest in respect to the mode of protection against x-irradiation injury. However, the mitochondria of only a limited number of x-irradiated mice treated with bone marrow were examined, and the comments in this respect are necessarily of preliminary nature.

The Mice and the Methods

Two hundred and forty-nine adult mice of strains C_3Hf, BALB/c, and $(C_{57}L \times A)F_1$ hybrid were x-irradiated, and 147 non-irradiated mice were studied. Ten of the x-irradiated and 8 of the non-irradiated specimens were lost by unsuccessful fixation of mitochondria, bringing the total studied cytologically to 378. Both male and female animals were included, and there was no morphologic evidence of

mitochondrial differences in respect to response to x-irradiation in relation to sex or strain. The age ranged from 3 to 4 months, and within this range no differences in mitochondria due to age were detectable after x-irradiation. No fetal, newborn, or senile mice were studied. The mice were fed Purina Chow with water ad libitum. The experiments were not statistically analyzed. The number of mice

TABLE I

Number of Mice in the Different Groups

	500 r.	900 r.	1,200 r.
Control animals: normal, fed, non-irradiated, water ad libitum	25	37	18
Non-irradiated, without food, water ad libitum until liver was excised 24 hours or more later	21	36	10
X-irradiated, fed, water ad libitum	22	90	35
X-irradiated, without food for 24 hours before irradiation, plus time after irradiation until liver was excised; water ad libitum	10	80	12

subjected to each set of environmental factors studied is shown in Table I.

Small numbers of mice (5 to 10) were irradiated at one time. The control animals also were handled in small groups. A piece of liver was excised from each mouse. In some instances, the mice were deprived of food for 24 hours prior to x-irradiation and then only water was given until the time of excision of the liver. The food was similarly withdrawn from non-irradiated mice to ascertain the fat accumulated in the liver as a result of inanition. The detailed description of the mitochondria of x-irradiated mice is confined to the liver of those animals that were given food and water throughout the experiment.

The physical factors for the irradiation were as follows: 186 KVP at 20 ma.; two tubes opposite each other were used with the mice placed in the center 54 cm. from each target; the beams were filtered by 0.25 mm. Cu and 0.55 mm. Al; the dose rate was approximately 96 r. per minute.

Fixation of Mitochondria

The mouse was given a solution of nembutal intraperitoneally sufficient to induce light anesthesia. Thin slices, 4 by 2 by 6 mm. in size, were excised from the middle of the largest lobe of the liver and blotted to remove excess blood. These were placed immediately in

various fixatives to preserve the minerals, chromatin, Golgi material, nucleoproteins, fats, and glycogen. However, the present observations deal only with the liver fixed and colored according to a modified method of Regaud[88] for mitochondria, which is as follows:

1. Place in cold (5° C.) Regaud's fluid
   3% potassium dichromate ..... 80 ml.
   40% formaldehyde ..... 20 ml.
   for 4 days in the refrigerator at 5° C.
   Change fluid every day.
2. Mordant for 8 days in 3% $K_2Cr_2O_7$ in the cold, changing the fluid every other day.
3. Wash and embed in paraffin.
4. Cut sections at 3, 4, 6, and 8 μ.
5. Mordant in iron alum and color in the usual way with Regaud's hematoxylin. No counter stain.

The Mitochondria of the Hepatic Cells

The Normal Hepatic Cell

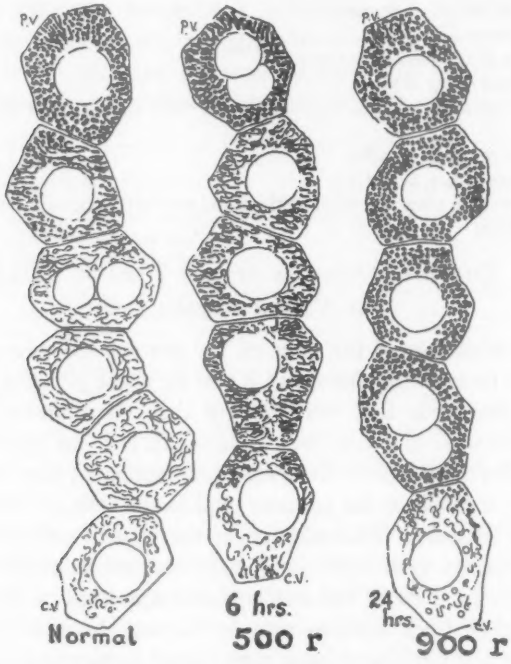
The mitochondria of the liver of the normal fed mice were found to be in the form of fine filaments, a few very fine globules, and mostly short and long rods that were thicker than the filaments. All three forms—filaments, globules, and rods—may and do appear in every cell including the cells of the peripheral region of the lobule at the portal vein, the cells of the midzone, and in the cells around the central vein. Fine filaments predominated in the central cells of the lobule, thicker filaments in the midzonal cells, and short, thick rods in the peripheral cells. Few of the mitochondria appeared as large globules. When serial or thick sections were examined, almost all of the mitochondria of the peripheral cells were found to be rod-shaped.

The filamentous and rod-shaped mitochondria of the midzonal cells were found to be polarized in perpendicular alignment to the blood-vascular surface of the cell (Text-fig. 1 and Fig. 1). The free end of the mitochondrion appeared to be directed toward the nuclear membrane, giving the cell the appearance of cross-striation. This alignment of mitochondria in the liver cell resembles that of the proximal pole of the cells of the convoluted portions of the uriniferous tubule of the kidney where the *bâtonnets* (mitochondria) appear like a bundle of sticks bunched against the blood-vascular surface of the cell.

500 r.

Twenty-two fed mice were exposed to 500 r. The liver was excised and fixed at 6 and 8 hours after irradiation. The distribution and form of mitochondria of only 10 of these mice were altered as compared

with the distribution and form of the mitochondria of the untreated fed mice. Many of the mitochondria were vesiculated and aligned against the vascular surface of the hepatic cell. This is shown in Figure 2 and depicted by free-hand drawing (Text-fig. 1). This mitochondrial



Text-figure 1. Free-hand drawings of mitochondria of liver cells of normal fed mice to show comparative changes following exposure to 500 r. and 900 r. total-body irradiation. Normal liver is shown in first column at left; liver at 6 hours after exposure to 500 r. in second column; and liver at 24 hours after exposure to 900 r. in third column at right. P.V. means portal vein; C.V. means central vein. The mitochondrial changes may not represent a consequential process, since some mice show the effect of exposure to x-ray more promptly than others. Of note is the alignment of mitochondria perpendicular to blood-vascular surfaces of the cell. Taken from Regaud preparations. 4 μ sections.

alteration involved chiefly the cells of the midzone of the lobule. The mitochondria of the perinuclear cytoplasm were essentially unaltered. It appeared that the mitochondrial filaments and rods were more densely packed in the peripheral cytoplasm of the hepatic cells in x-irradiated than in normal mice. It was apparent that in this material there was no evidence whatsoever to indicate whether new mitochondria were being formed from substances absorbed from the

blood stream or whether the cytoplasm was forming new mitochondria to be released either to the blood stream or to the internal cytoplasm.

900 r.

The state of the mitochondria of the liver of fed mice exposed to 900 r. irradiation was strikingly different than that at the lower exposure of 500 r. Of 42 animals examined 6 to 8 hours after exposure to 900 r., 38 livers revealed evidence of vesiculating, fragmenting, and globulating mitochondria in the midzonal cells and cells of the peripheral portal zone. This alteration is shown in Text-figure 1 and Figure 3. It is notable that none of these mice showed grossly detectable evidence of damage to living processes as determined by outward appearances and behavior at 6 to 8 hours after irradiation.

At 24 hours, in 26 of 38 mice irradiated, the mitochondria were fragmented and the cells showed widespread evidence of the globulating process (Fig. 4). At this time the animals appeared to be injured and remained quiet in the cage.

At 48 hours after 900 r. irradiation, the mitochondria were globulated in 4 of 6 animals exposed. Only 4 mice were examined at 96 hours after 900 r. irradiation, and the livers of all of them were excised when they appeared to be moribund. The mitochondria in such a state were not only globulated and fragmented but also markedly diminished in mass and present for the most part in the peripheral cytoplasm.

1,200 r.

Only 35 fed mice were exposed to 1,200 r. irradiation. The livers of all of these animals, including 12 without food, revealed mitochondrial alterations. Of 12 fed, x-irradiated mice examined 6 to 8 hours after irradiation, the mitochondria of the livers of all of them were globulated from the peripheral portal zone through the cells of the midzone of the lobule. They were present chiefly in the peripheral cytoplasm against the vascular surface of the cell (Text-fig. 2 and Fig. 5).

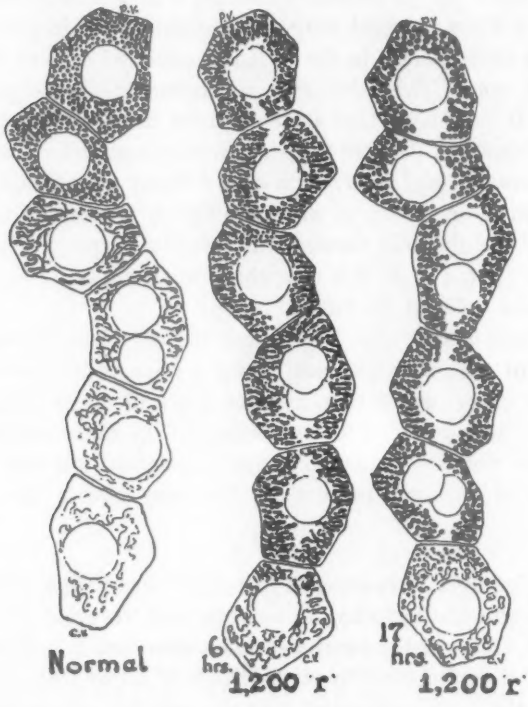
The mitochondria of one of these specimens taken at 48 hours after irradiation, when the mouse was apparently near death, were globulated and thick as in Figure 6. These were distributed against the vascular wall of the cell, and this state existed throughout the specimen of liver, sectioned serially.

The Intranuclear Bodies

It was in such a state as was found at 8 hours after 1,200 r. that the hepatic cell possessed several intranuclear bodies (nucleoli?)

(Fig. 7) which appeared to be constructed of a large, central, red core with one or two or more green satellites, as revealed by Pappenheim's methyl green-pyronine method. These intranuclear bodies were numerous in all livers of x-irradiated mice, but they were present and less numerous in livers of normal mice.

At 24 hours after 1,200 r., the mitochondria of all 6 mice examined



Text-figure 2. Free-hand drawings of hepatic cells of normal mice to show comparative changes in mitochondria of livers of different mice following exposure to 1,200 r. total-body irradiation. Normal liver is shown in first column at left; liver at 6 hours after exposure to 1,200 r. in second column; and liver at 17 hours after exposure to 1,200 r. in third column. P.V. means portal vein; C.V. means central vein. Of note are the fragmentation and globulation of the mitochondria. Taken from Regaud preparations. 4 μ sections.

were globulated and distributed at the periphery of the cytoplasm as shown in Figure 8, which was taken at 24 hours while the mouse was still relatively active.

At 48 hours after 1,200 r., the livers of 6 animals examined possessed globulated mitochondria aligned perpendicular to the vascular surface of the cell, similar to those found at 8 hours.

At later periods after x-irradiation, the mitochondria of all the mice studied seemed to be diminished in number and distributed at the blood-vascular surface of the cell. Figure 9 shows the mitochondria in the midzonal cells at 72 hours after 1,200 r., and Figure 10 shows them in the midzonal cells at 96 hours (when this particular animal was moribund). These very late changes do not seem to be significant from the standpoint of this report since it is probable that they were concerned with cellular degeneration in moribund animals.

Influence of Bone Marrow on Mitochondria

The femoral and humeral bone marrow from a normal mouse was injected intravenously into an x-irradiated mouse within a short period after total-body exposure. Only a very few mice have been so treated, and they were killed at various intervals following such treatment as

TABLE II

Number of Mice Given Bone Marrow after X-Irradiation, Showing Time after Irradiation When Liver Was Excised

	900 r.		1,200 r.
X-irradiated, fed, and bone-marrow injected intravenously; the liver was excised at time indicated after x-irradiation	24 hrs.	2	2
	48 hrs.	6	6
	72 hrs.		6
	96 hrs.	4	2
	120 hrs.	2	
	144 hrs.	2	
	168 hrs.	2	
	188 hrs.	2	
	12 days	1	
X-irradiated and fed; no bone marrow	24 hrs.	2	2
	48 hrs.	6	6
	72 hrs.	2	6
	96 hrs.	4	2
	120 hrs.	2	
	144 hrs.	2	
	168 hrs.	2	
	188 hrs.	2	

indicated in Table II. In spite of the limits of this experiment, it may be of value to record the results without comment on their physiologic significance.

The mitochondria of the livers of 4 mice at 24 hours after such treatment with bone marrow following 900 r. or 1,200 r. were globulated throughout the liver lobule. The mitochondria appeared to be little different at this time than at 24 hours following x-irradiation alone. However, the livers of 2 mice killed at 96 hours after receiving bone marrow following 1,200 r. were rich in mitochondrial elements, particularly in the peripheral portal region. The mitochondria were

chiefly in the form of short rods and distributed throughout the cytoplasm. The mitochondria of the central cells were almost all rod-shaped or globular. These animals were active, and had eaten food, as indicated by the contents of the stomach at necropsy. On the other hand, 4 mice at 48 hours following 1,200 r. and bone-marrow therapy showed mitochondria which were globulated and polarized against the vascular surface of the cell, as in mice treated with x-ray alone.

None of the mice exposed to 1,200 r. and treated with bone marrow appeared to exhibit a completely normal state of the mitochondria. Bone marrow appeared to have a more profound influence on the form of mitochondria in the liver after 900 r. irradiation than after 1,200 r. The mitochondria of the livers of 4 mice at 96 hours and of one mouse at 12 days after 900 r. seemed to have recaptured their normal state, although more of them were globulated than in non-irradiated mice, and the cells in the peripheral portal zone were not as rich in mitochondria as those of non-irradiated mice. This latter liver also contained some neutral fat beyond the usual amount, but not more than the livers of some normal mice.

As previously stated, these observations are scarcely more than suggestive that injection of bone marrow may affect the state of the mitochondria of the liver. Although it is true that all mice exposed to 1,200 r. irradiation may be expected after 96 hours to be damaged beyond recovery, it must be emphasized that only one mouse was observed in the present experiment, and that no information is available to show the effect on mitochondria of injecting bone marrow at various intervals of time following x-irradiation.

Discussion

These observations show that the mitochondria in the hepatic cells respond promptly in nearly all mice exposed to total-body x-irradiation of lethal magnitude. It is not unreasonable to assume, on account of biologic differences, that the animals that did not reveal mitochondrial alterations at 8 hours after irradiation might have developed the same changes in delayed reaction, and possibly might have been destined to survive for a longer period than the others. The mitochondria of the liver are only slightly affected 8 hours after 500 r. total-body irradiation, being simply distributed at the blood vascular surface of the hepatic cell; whereas 900 r. or 1,200 r., highly lethal exposures, induce globulation and fragmentation of the mitochondria and relocation of them at the peripheral cytoplasm in less than 8 hours.

The significance of this is by no means to be determined from the

present data. Why do the mitochondria appear so richly oriented perpendicular to the blood vascular surface of the cell, while the perinuclear cytoplasm is relatively poor in mitochondria? Is it in response to loss of phospholipids in other vital tissues? It is not known whether mitochondria would respond in this way to x-irradiation of the liver alone in shielded experiments.

An important function of the liver is the formation of phospholipids that are evidently stored as mitochondria to be released to the blood stream, and the mitochondria of the liver do respond directly or indirectly to total-body irradiation in mice. It should be remarked especially that other agents, such as heat, cause alterations of the mitochondria of the liver similar to those seen after x-irradiation, without killing the tissue.

The mitochondria of the midzonal hepatic cells of the hepatic lobule seem to be the first to respond by globulation and increased polarization against the blood-vascular surface of the cell. These mitochondria are in the form of short and long, thick rods, and they change by globulation and fragmentation. The fine filamentous mitochondria of the perinuclear cytoplasm of these cells and of the centrally located cells become vesiculated and beaded in a much different manner at a later time following x-irradiation. Perhaps this may, upon closer inspection, indicate that the hepatic chondriome constitutes different types of mitochondria that respond differently to the same circumstance (*mixochondriome*). A topographic study of other elements of the cell, such as calcium, magnesium, and iron, following x-irradiation may be of value.

Summary

The mitochondria of the hepatic cells were globulated, fragmented, vesiculated, and polarized in alignment perpendicular to the blood-vascular surface of the cell in the liver at 6 to 8 hours following exposure of the entire body to x-irradiation equivalent to 500, 900, or 1,200 r. units in nearly all of 239 C3Hf, BALB/c, and (C57L x A)F_1 hybrid mice. It is believed that this alteration of the mitochondria represents an early response to irradiation injury, but that it is not peculiar to x-irradiation injury since other factors, such as heat, may induce similar changes. These initial mitochondrial changes probably occur in the livers of all mice at some variable time following x-irradiation, regardless of whether or not the mouse survives the exposure to x-rays. The different responses of mitochondria in relation to time after x-irradiation may represent reactions of different types of mitochondria.

REFERENCES

1. Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Veit & Co., Leipzig, 1890, 145 pp.
2. Valette St. George, v. la. Ueber die Genese der Samenkörper. *Arch. f. mikr. Anat.*, 1865, 1, 403–414.
3. Flemming, W. Beiträge zur Kenntniss der Zelle und ihrer Lebenserscheinungen. *Arch. f. mikr. Anat.*, 1879, 16, 302–436.
4. Fischer, A. Fixirung, Färbung und Bau des Protoplasmas. G. Fischer, Jena, 1899, 362 pp.
5. Verworn, M. Allgemeine Physiologie. G. Fischer, Jena, 1895, 584 pp.
6. Bütschli, O. Ueber den Bau der Bacterien und verwandter Organismen. C. F. Winter, Leipzig, 1890, 37 pp.
7. Galeotti, G. Ueber die Granulationen in den Zellen. *Internat. Monatschr. f. Anat. u. Physiol.*, 1895, 12, 440–460.
8. Benda, C. Ueber die Spermatogenese der Vertebraten und höherer Evertebraten. II. Die Histiogenese der Spermien. *Arch. f. Anat. u. Physiol.* (physiol. Abth.), 1898, pp. 393–398.
9. Michaelis, L. Die vitale Färbung, eine Darstellungsmethode der Zellgranula. *Arch. f. mikr. Anat.*, 1899–1900, 55, 558–575.
10. Fauré-Fremiet, E. Étude sur les mitochondries des Protozoaires et des cellules sexuelles. *Arch. d'anat. micr.*, 1909–10, 11, 457–648.
11. Bensley, R. R. Studies on the pancreas of the guinea pig. *Am. J. Anat.*, 1911–12, 12, 297–388.
12. Cowdry, E. V. Mitochondria and other cytoplasmic constituents of the spinal ganglion cells of the pigeon. *Anat. Rec.*, 1912, 6, 33–38.
13. Lewis, M. R., and Lewis, W. H. Mitochondria (and other cytoplasmic structures) in tissue cultures. *Am. J. Anat.*, 1914–15, 17, 339–401.
14. Mathews, A. P. Physiological Chemistry. William Wood & Co., New York, 1930, ed. 5, 1233 pp.
15. Kingsbury, B. F. Cytoplasmic fixation. *Anat. Rec.*, 1912, 6, 39–49.
16. Fauré-Fremiet, E. Sur la constitution des mitochondries des gonocytes de l'*Ascaris megalocephala*. *Compt. rend. Soc. de biol.*, 1912, 72, 346–347.
17. Mayer, A.; Rathery, F., and Schaeffer, G. Les granulations ou mitochondries de la cellule hépatique. *J. de physiol. et de path. gén.*, 1914–15, 16, 581–596, 607–622.
18. Löwschin, A. M. "Myelinformen" und Chondriosomen. *Ber. d. deutsch. bot. Gesellsch.*, 1913, 31, 203–209.
19. Bensley, R. R., and Hoerr, N. L. Studies on cell structure by the freezing-drying method. VI. The preparation and properties of mitochondria. *Anat. Rec.*, 1934, 60, 449–455.
20. Kögel, G. Über die Röntgenstrahlenempfindlichkeit des Lecithins und den Einschlagort der X-Strahlen im Eiweissmoleköl. *Ztschr. f. Krebsforsch.*, 1937–38, 47, 169–175.
21. Potter, R. L., and Bethell, F. H. Oxidative phosphorylation in spleen mitochondria. *Federation Proc.*, 1952, 11, 270.
22. Beckton, H., and Colwell, H. A. A note on the effects of various reagents on the demonstrability of Altmann's granules. *Arch. Middlesex Hosp.*, 1911, 23, 52–55.

23. Beckton, H., and Russ, S. The effect of radium emanation on Altmann's granules. *Arch. Middlesex Hosp.*, 1911, **23**, 99–105.

24. Entenman, C., and Weinman, E. O. Effect of x-irradiation on incorporation of inorganic P^{32} into phospholipids by rat liver slices. *Federation Proc.*, 1952, 11, 44.

25. Gatenby, J. B.; Mukerji, R. N., and Wigoder, S. B. The effect of x-radiation on the spermatogenesis of *Abraxas grossulariata*. *Proc. Roy. Soc., London, s. B.*, 1929–30, **105**, 446–469.

26. Hirsch, G. C. Analyse der Restitution des Sekretmaterials im Pankreas mittels Röntgenstrahlen. Beobachtungen an lebenden Zellen. *Arch. f. Entwcklngsmechn. d. Organ.*, 1931, **123**, 792–821.

27. Bloom, W. Histopathology of Irradiation from External and Internal Sources. McGraw-Hill Book Co., Inc., New York, 1948, 808 pp.

28. Bloom, W., and Bloom, M. A. Histological Changes after Irradiation. In: Radiation Biology, Hollaender, A. (ed.). McGraw-Hill Book Co., Inc., New York, 1954, 1, Pt. 2, 1091–1143.

29. Tschassownikow, N. Ueber die Wirkung der Röntgenstrahlen auf den feinen Bau der Leberzellen beim Frosch. *Virchows Arch. f. path. Anat.*, 1928, **269**, 166–177.

30. Heilbrunn, L. V., and Mazia, D. The Action of Radiations on Living Protoplasm. In: Biological Effects of Radiation, Duggar, B. M. (ed.). McGraw-Hill Book Co., Inc., New York, 1936, 1, 625–676.

31. Heilbrunn, L. V., and Young, R. A. Indirect effects of radiation on sea urchin eggs. *Biol. Bull.*, 1935, **69**, 274–278.

32. Congdon, C. C.; Uphoff, D., and Lorenz, E. Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: a histopathologic study. *J. Nat. Cancer Inst.*, 1952–53, **13**, 73–107.

33. Duryee, W. R. The nature of radiation injury to amphibian cell nuclei. *J. Nat. Cancer Inst.*, 1949–50, **10**, 735–795.

34. Wilson, W. L. The effect of roentgen rays on protoplasmic viscosity changes during mitosis. *Protoplasma*, 1949–50, **39**, 305–317.

35. Policard, A. Sensibilité des chondriosomes aux élévations de température. *Compt. rend. Soc. de biol.*, 1912, **72**, 228–229.

36. MacCardle, R. C. The effect of temperature on mitochondria in liver and kidney cells of fish. *Anat. Rec.*, 1931, suppl. **51**, 75.

37. MacCardle, R. C. The effect of temperature on mitochondria in liver cells of fish. *J. Morphol.*, 1937, **61**, 613–639.

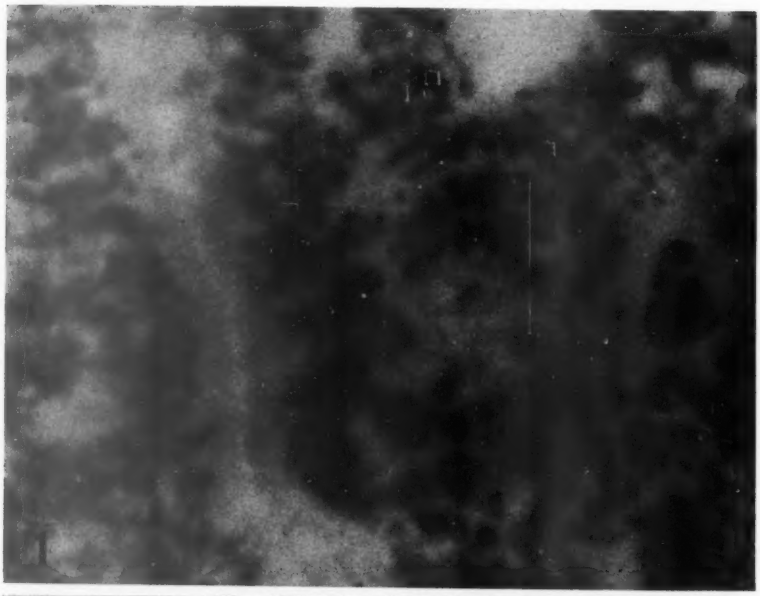
38. Regaud, C. Études sur la structure des tubes séminifères et sur la spermatogénèse chez les mammifères. *Arch. d'anat. micr.*, 1909–10, 11, 291–431.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Normal liver of fed, adult, male mouse. Mitochondria of hepatic cell of midzone (intermediate zone) of lobule. Fine filaments and rods may be seen in the cell in the center of the photomicrograph. Mitochondria are polarized in perpendicular alignment against the blood-vascular surface of the cell. The heavy black dots at the right are red blood cells in a sinusoid. Regaud's hematoxylin. 4 μ section. Photomicrograph taken with Bausch and Lomb oil-immersion 120 $\times$ objective. Total magnification on printed page is approximately $\times$ 4,600.

FIG. 2. 500 r. at 8 hours after x-irradiation. Liver of fed, adult, male mouse. Midzonal cells. There is perpendicular alignment of the mitochondria at the blood-vascular surface of a cell. Large, black bodies are red blood cells in a sinusoid. Regaud's hematoxylin. 4 μ section. Photomicrograph taken with Leitz oil-immersion 114 $\times$ objective. Total magnification on printed page is approximately $\times$ 3,000.



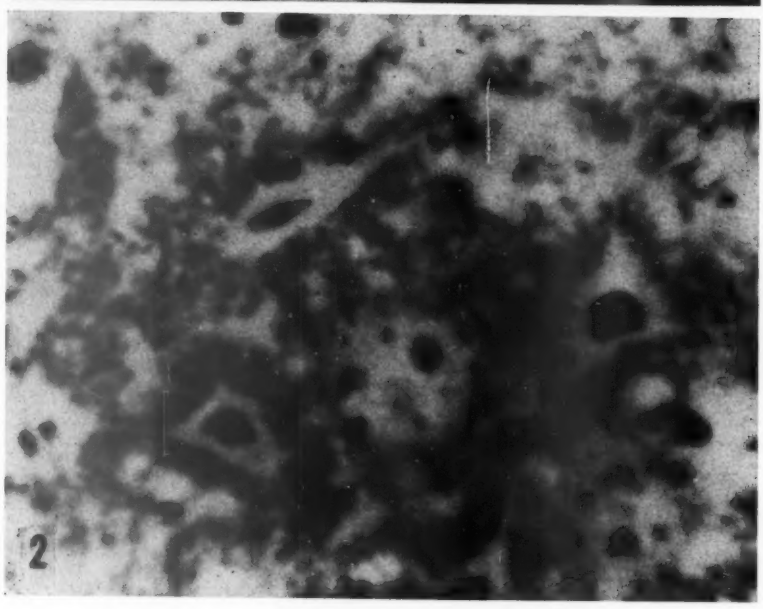

2

FIG. 3. 900 r. at 6 hours after x-irradiation. Liver of fed, adult, male mouse. Mitochondria of midzonal cells are globulated, fragmented, and vesiculated. The cell of the central zone near the central vein at the right possesses fine filamentous and finely globular mitochondria. Regaud's hematoxylin. 4 μ section. Taken with Leitz oil-immersion 114 $\times$ objective. Total magnification on printed page is approximately $\times$ 3,000.

FIG. 4. 900 r. at 24 hours after x-irradiation. Liver of fed, adult, male mouse. Mitochondria of midzonal cells are globulated and fragmented, and aligned perpendicular to the blood-vascular surface of the cell. Regaud's hematoxylin. 4 μ section. Taken with Leitz 114 $\times$ oil-immersion objective. Total magnification on printed page is approximately $\times$ 4,000.

FIG. 5. 1,200 r. at 6 hours after x-irradiation. Liver of fed, adult, mouse. The peripheral cytoplasm is rich in globulated mitochondria aligned against the blood vascular surface of the cell. The mitochondria of a centrally located cell at the lower right corner also are globulated. Perinuclear cytoplasm free of mitochondria. Regaud's hematoxylin. 4 μ section. Taken with Leitz oil-immersion 100 $\times$ objective. Magnification on printed page is approximately $\times$ 2,200.

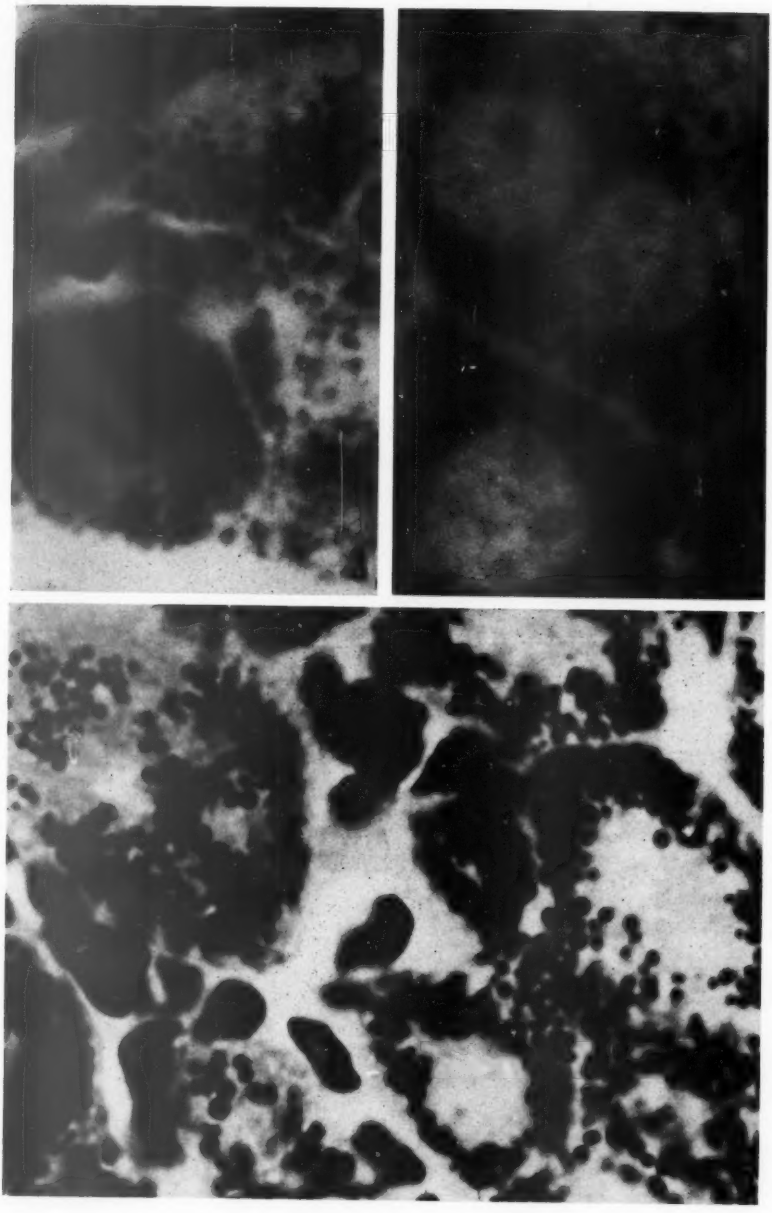


FIG. 6. 1,200 r. at 48 hours after irradiation. Liver of x-irradiated, fed, adult, male mouse that was moribund at the time a piece of liver was excised. Mitochondria are globulated and fragmented in the peripheral cytoplasm of midzonal cells of a lobule. Of note is polarization perpendicular to blood-vascular surface; perinuclear cytoplasm is free of mitochondria. Regaud's hematoxylin. 4 μ section. Taken with a Bausch and Lomb oil-immersion 97 $\times$ objective. Magnification on printed page is approximately $\times$ 4,000.

FIG. 7. 1,200 r. at 8 hours after x-irradiation, showing intranuclear bodies (nucleoli?) in midzonal cells of the liver of x-irradiated, fed, adult, male mouse. These intranuclear forms occur also in normal mice. Regaud's hematoxylin. 4 μ section. Taken with Bausch and Lomb 97 $\times$ oil-immersion objective. Magnification on printed page is approximately $\times$ 4,800.

FIG. 8. 1,200 r. at 24 hours after x-irradiation. Midzonal cells of liver of x-irradiated, fed, adult, male mouse. Mitochondria are globulated and fragmented and aligned in peripheral cytoplasm. Regaud's hematoxylin. 4 μ section. Taken with Bausch and Lomb 97 $\times$ oil-immersion objective. Magnification on printed page is approximately $\times$ 4,640.

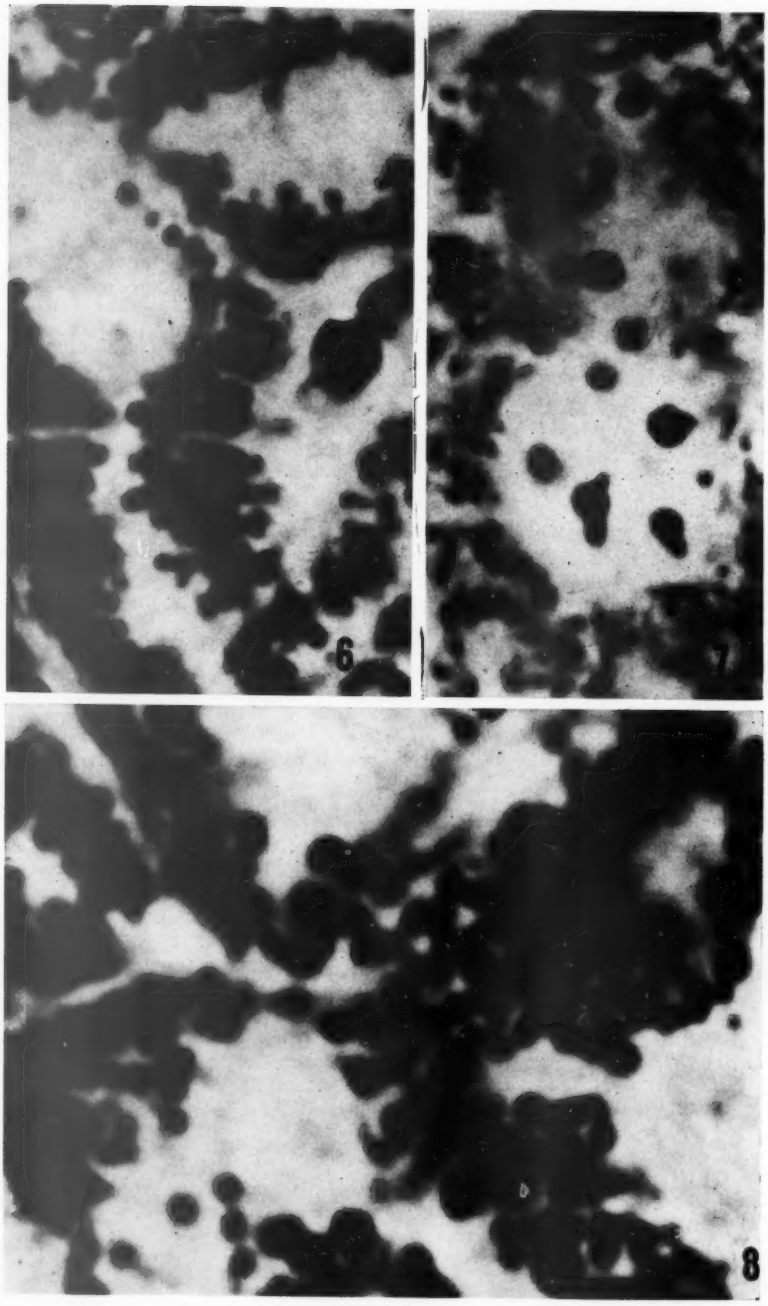
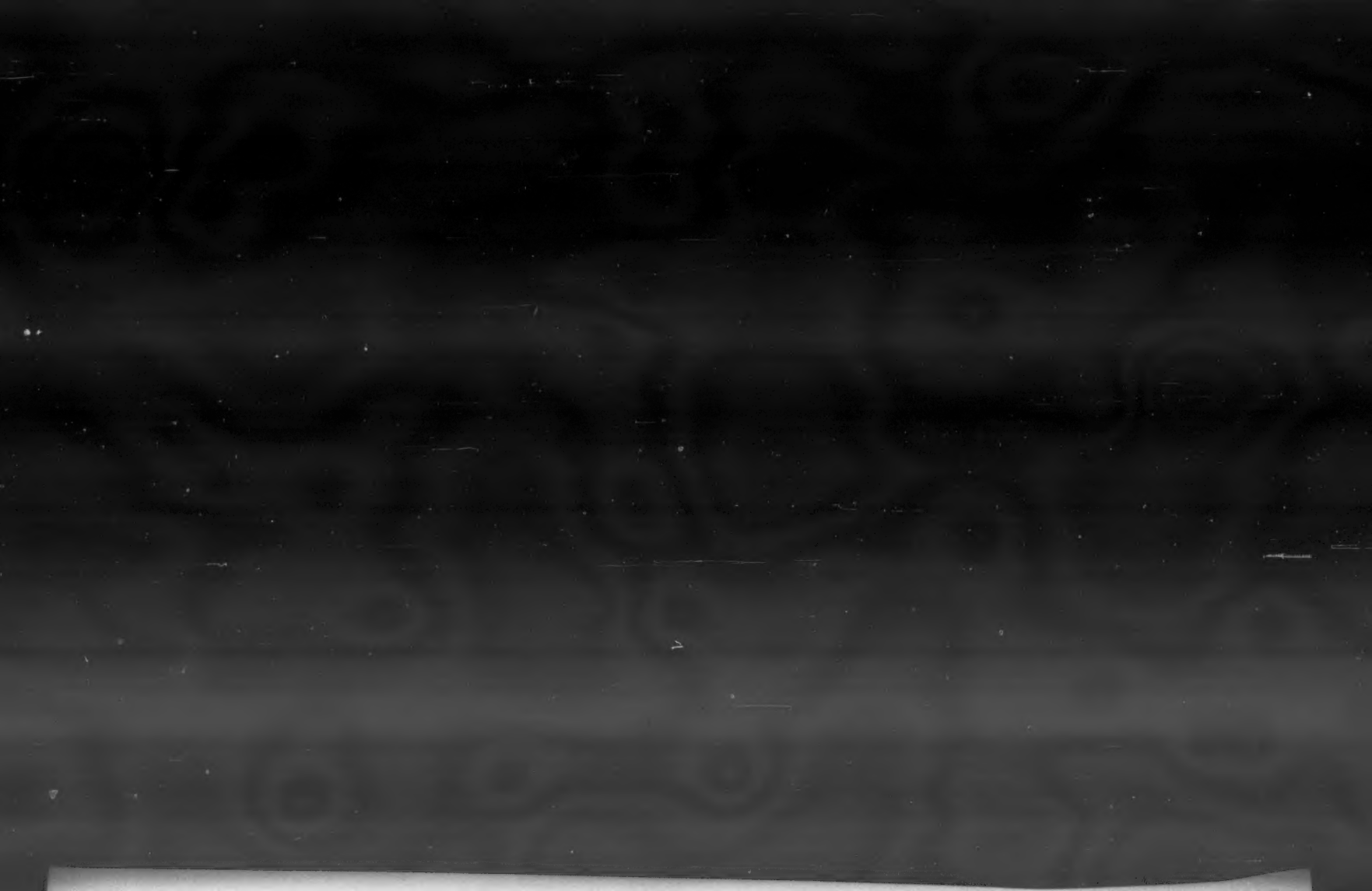
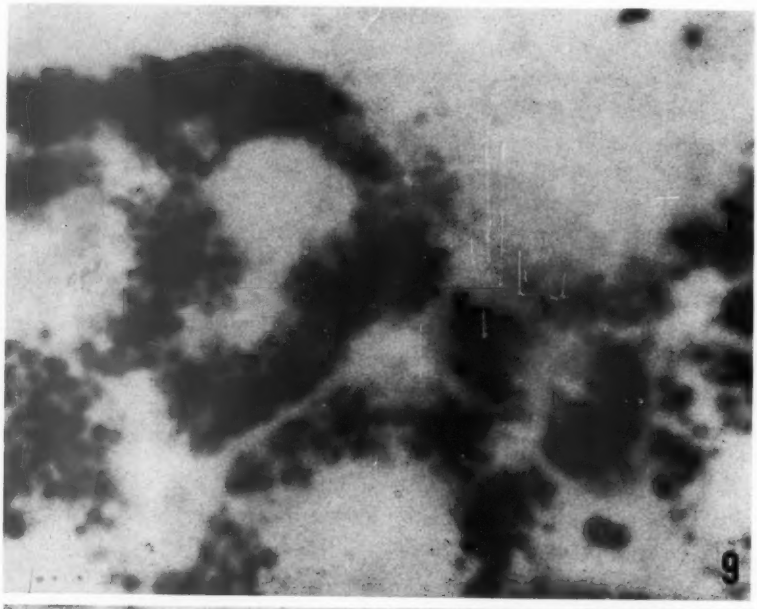
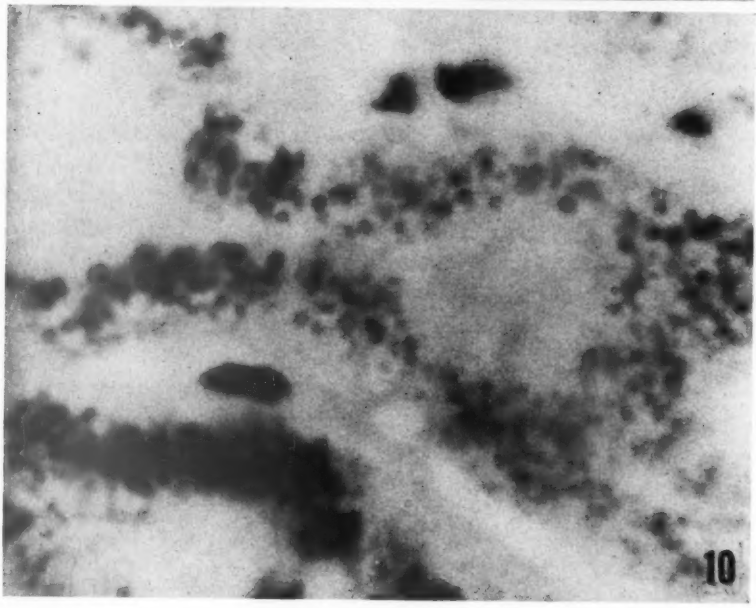

6
7
8

FIG. 9. 1,200 r. at 72 hours after irradiation. Midzonal cells of the liver of an x-irradiated, fed, adult, male mouse. Mitochondria are present as small droplets, globulated and vesiculated. Peripheral cytoplasm is rich in mitochondria while perinuclear cytoplasm is devoid of them. Regaud's hematoxylin. 4 μ section. Taken with 100 $\times$ Leitz oil-immersion objective. Magnification on printed page is approximately $\times$ 4,600.

FIG. 10. 1,200 r. at 98 hours after irradiation. Liver of moribund, adult, male mouse. Midzonal cells. Mitochondria are diminished and globulated, aligned toward vascular wall. A large central cell near a central vein at right center shows finer globules and vesiculated mitochondria. Regaud's hematoxylin. 4 μ section. Taken with 120 $\times$ Bausch and Lomb oil-immersion objective. Magnification on printed page is approximately $\times$ 4,600.




9


10

NEONATAL JAUNDICE WITH GIANT CELL TRANSFORMATION OF THE HEPATIC PARENCHYMA *

H. F. SMETANA, M.D., and F. B. JOHNSON, M.D.
From the Armed Forces Institute of Pathology, Washington 25, D.C.

A study of the pathogenesis of jaundice in the neonatal period was undertaken with a view to determining the nature of the underlying pathologic changes in the cases of this kind on file at the Armed Forces Institute of Pathology.[1] After eliminating cases of icterus neonatorum and of jaundice due to erythroblastosis fetalis, there remained a group of 37 infants whose clinical history and laboratory findings suggested atresia of the biliary passages. Anatomical evidence of atresia of various portions of the extrahepatic biliary system, however, was found in only 21 instances, in each of which significant intrahepatic changes also were present. In 2 cases there was cyst formation of the common bile duct. Atresia of portions of the duodenum accounted for ostensible biliary atresia in 3 instances, complicated by intrahepatic changes in 2. Intrahepatic abnormalities were responsible for the clinical picture of obstructive jaundice in 11 cases in which the extrahepatic biliary passages appeared normal. Giant cell transformation of the hepatic parenchyma was observed in 8 of these 11 cases, evidence of intrahepatic atresia of biliary passages in 2, and abnormal bile canaliculi of embryonal character, distended by partly inspissated bile, in one.

Of particular interest was apparent clinical biliary atresia associated with giant cell transformation of the hepatic parenchyma. Three of the 11 cases in which this feature was observed also exhibited extrahepatic atresia. The usual clinical picture was that of jaundice which appeared shortly after birth and increased steadily. The stools were light in color, pasty, and devoid of bilirubin. High levels of serum bilirubin were demonstrated in direct and indirect van den Bergh tests, while urinary urobilinogen was usually absent; serum phosphatase values were increased and the results of liver function tests suggested hepatic damage of varying degree.

Many of the records stressed the paucity of clinical symptoms despite the severe and progressive icterus. Death usually occurred within a few weeks after birth, although some of the infants lived for many months.

* Received for publication, December 8, 1954.

At necropsy the liver was enlarged; its surface, as a rule, was smooth; and on cut surfaces the parenchyma was deeply jaundiced. In cases of prolonged disease the organ showed diffuse scarring. The extrahepatic biliary passages were normal in 8 cases but were always devoid of bile. In one case there was complete atresia of all extrahepatic bile ducts; in another, there was obliteration of the cystic duct only; in a third, the pathologic changes seen at necropsy were interpreted as cyst formation of the common bile duct.

Microscopically, the general lobular architecture was somewhat obscure, but efferent veins and portal canals could be distinguished. All hepatic cell columns were replaced by giant liver cells radiating from the efferent vein toward the periphery of the lobules (Fig. 1). Individual giant cells measured as much as 400 μ in diameter and contained as many as thirty nuclei. These cells were flanked by sinusoids which often seemed narrow and compressed (Fig. 2). Most of the portal canals showed moderate infiltrates consisting of lymphocytes and monocytes. In occasional instances the portal triads exhibited large lymphatics and rather immature bile channels with irregular lumina which were lined by prominent epithelial cells (Fig. 3). The cytoplasm of most of the giant liver cells contained tiny particles of bile (Fig. 4); iron pigment varied in amount from case to case, and fat and lipofuscin could be demonstrated only occasionally. The Kupffer cells also contained particles of bile and sometimes iron pigment in varying quantities, but neither fat nor lipofuscin. Bile was not observed in the lumina of the intralobular bile ducts or in the larger bile passages. The intralobular bile ducts in some cases seemed disoriented and coursed aimlessly through the lobules.

Reticulum fibers were found to surround the columns of giant liver cells, separating them from the sinusoids. Islets of extramedullary hematopoiesis were scattered through the lobules, especially in the livers of infants who had died during the early post-natal period. The branches of the portal vein, hepatic artery, and hepatic vein appeared unaltered.

Evidence of formation of regenerative nodules of liver cells was not seen when hepatic tissue was obtained for biopsy from infants suffering from this condition or at post-mortem examination. In cases of prolonged survival the hepatic tissue usually showed diffuse scarring (Fig. 5). The number of individual giant liver cells appeared to be reduced, a change attributed to destruction and disintegration of these cells. Diminution in giant liver cells was accompanied by an inflammatory infiltration composed of lymphocytes, monocytes, and

polymorphonuclear leukocytes (Fig. 6). Actual formation or regeneration of the giant liver cells could never be detected.

The disintegration of liver cells without subsequent regeneration led to a steady diminution in their number, accompanied by an apparent increase in stroma resulting from condensation and collapse of reticulum and collagenous connective tissue fibers surrounding islets of surviving giant liver cells (Fig. 7). The final picture was one of diffuse fibrosis of the liver with complete disorganization of any lobular structure of the remaining giant liver cell elements (Fig. 8).

Although evidence of portal hypertension could be found at this stage, death was usually attributable to hepatic insufficiency. Except for jaundice of all tissues, there were no other pertinent or constant pathologic changes in the viscera.

Discussion

The alterations of the liver seen in cases of jaundice of the neonatal period with giant cell transformation of the liver have been attributed to viral hepatitis contracted by the fetus from the mother during gestation.[2,3] Craig and Landing,[2] in particular, have asserted that the histologic changes seen in this condition conform in all respects to the criteria of viral hepatitis as postulated by Weisbrod and co-workers.[4] It would have to be assumed, however, that the fetal liver reacts uniquely to the virus of infectious hepatitis, for the actual histopathologic changes seen in this condition bear but little resemblance to those observed in hepatitis occurring in the post-natal period: there are no balloon cells, no acidophilic bodies, no widespread necrosis of individual liver cells followed by regeneration, and monocytic infiltrates within the lobules and in the portal triads are either absent or poorly developed.[5] On the other hand, although occasional giant liver cells may occur in viral hepatitis, the massive and general transformation of liver parenchyma into giant cells is not seen in post-natal viral hepatitis. Presence of bile in liver cells and Kupffer cells is likewise not a feature in viral hepatitis, although bile thrombi may be seen in intercellular bile canaliculi. Clinically, the lack of bile in the intestinal contents and the corresponding absence of urobilinogen are certainly not characteristic of post-natal viral hepatitis.

The microscopic features considered most significant and characteristic of this condition are the multinucleated giant liver cells containing tiny bile particles in their cytoplasm, presence of bile in Kupffer cells, and absence of intercellular bile capillaries. It is this last abnormality that probably is the key to the understanding of the histogenesis of

this condition: although bile is being secreted, it does not reach the efferent biliary channels but is retained within the liver cells, where, perhaps, it exerts an undue stimulus that leads eventually to their destruction. The bile, abnormally liberated from such destroyed cells, reaches the sinusoids where it is, in part, phagocytized by Kupffer cells but chiefly is responsible for the production of generalized jaundice.

The pathogenesis of this remarkable lesion is not known. The influence of viral hepatitis of the mother on the fetus during pregnancy has not been proved beyond doubt, even though the virus of serum hepatitis has successfully been transmitted to volunteers from an infant and from its mother in one case.[6,7]

Dible[8] assumed that the neonatal condition is caused by the virus of hepatitis although he did not present evidence either that hepatitis occurred in the mother during pregnancy or that the virus of hepatitis was present in the mother's blood. A similar infectious background is assumed by other authors.[8,9] Martini,[10] on the other hand, in reporting a series of 57 cases of viral hepatitis occurring in mothers during pregnancy, observed that, though premature birth is perhaps more common under such circumstances, transmission of the virus to the fetus cannot be proved with certainty. According to his report, the chance of malformation of the child as a consequence of hepatitis of the mother during pregnancy is also remote. However, the possibility of a relationship between the virus of hepatitis of the mother and malformation of the fetus, as in German measles of the mother during pregnancy, cannot be entirely disregarded; further observations are needed to clarify this point. The association of the giant cell transformation of the liver cells with malformations of the extrahepatic bile ducts suggests a possible pathogenetic relationship.

The time of actual onset of the giant cell transformation of the liver is not known. Although the jaundice usually makes its appearance shortly after birth, its clinical onset may be related to the greatly augmented physiologic activity of the liver after delivery. On the basis of both biopsy and necropsy material from even the youngest infants, the transformation of the liver parenchyma into giant liver cells appears to have been completed *in utero*.

Until the background of this challenging condition is thoroughly explored and understood, the changes referred to as hepatitis in the neonatal period are perhaps best interpreted as aplasia of bile capillaries and as malformation of liver cells of unknown etiology.

Conclusions

The histopathologic changes in the liver most commonly seen in cases of hepatitis in the neonatal period are replacement of normal liver cells by multinucleated giant liver cells, lack of intercellular bile canaliculi, and absence of bile from the efferent biliary passages and the intestine; bile is being formed, however, and it accumulates within the giant liver cells and Kupffer cells.

Attribution of this condition to viral hepatitis occurring in the mother during pregnancy and transmitted to the fetus is highly speculative; instead, the clinical picture and the histopathologic changes are interpreted as consequences of the failure of development of the intercellular bile canaliculi which normally link the liver cells with the efferent bile passages; the association with malformations of the extrahepatic biliary passages would seem to strengthen this thesis. The cause of this maldevelopment is as yet unknown.

Pending final clarification, it is suggested that this condition be designated as jaundice of the neonatal period with giant cell transformation of liver cells.

REFERENCES

1. Smetana, H. F., and Stowens, D. Study of the pathogenesis of congenital biliary atresia. (In preparation.)
2. Craig, J. M., and Landing, B. H. Form of hepatitis in neonatal period simulating biliary atresia. *A. M. A. Arch. Path.*, 1952, **54**, 321–333.
3. Dible, J. H. Foetal and neonatal hepatitis and its sequelae. *Schweiz. Ztschr. f. Path. u. Bakt.*, 1953, **16**, 389–391.
4. Weisbrod, F. G.; Schiff, L.; Gall, E. A.; Cleveland, F. P., and Berman, J. R. Needle biopsy of the liver. III. Experiences in the differential diagnosis of jaundice. *Gastroenterology*, 1950, **14**, 56–72.
5. Smetana, H. F. The histologic diagnosis of viral hepatitis by needle biopsy. *Gastroenterology*, 1954, **26**, 612–625.
6. Stokes, J., Jr.; Wolman, I. J.; Blanchard, M. C., and Farquhar, J. D. Virus hepatitis in the newborn: clinical features, epidemiology, and pathology. *Am. J. Dis. Child.*, 1951, **82**, 213–216.
7. Stokes, J., Jr. Studies on Virus Hepatitis. Alpha Omega Alpha Lecture, Harvard Medical School, Boston, Mass., March 20, 1952.
8. Harris, R. C.; Andersen, D. H., and Day, R. L. Obstructive jaundice in infants with normal biliary tree. *Pediatrics*, 1954, **13**, 293–307.
9. Scott, R. B.; Wilkins, W., and Kessler, A. Viral hepatitis in early infancy. Report of three fatal cases in siblings simulating biliary atresia. *Pediatrics*, 1954, **13**, 447–452.
10. Martini, G. A. Hepatitis und Schwangerschaft. *Schweiz. Ztschr. f. Path. u. Bakt.*, 1953, **16**, 475–482.

[*Illustrations follow*]

LEGENDS FOR FIGURES

Jaundice of the neonatal period. Congenital anomaly of liver cells and bile canaliculi.

FIG. 1. Armed Forces Institute of Pathology accession no. 520738. The liver lobules are taken up by multinucleated giant liver cells; the portal areas are small and lightly infiltrated with lymphocytes and monocytes; the efferent veins are in proper position; the sinusoids are greatly narrowed. × 50.

FIG. 2. A.F.I.P. Acc. 520738. Multinucleated giant liver cells occupy the entire width of the columns. Their cytoplasm contains tiny particles of bile. Intercellular canaliculi are not evident. The sinusoids are narrow. Some of the Kupffer cells contain bile. × 360.

FIG. 3. A.F.I.P. Acc. 520738. Portal canal showing cross sections of immature appearing bile ducts with prominent lining cells, irregular lumina, and imperfect walls (D). The stroma of the portal field is moderately infiltrated with lymphocytes. The lymphatic channels (L) are greatly dilated. × 160.

FIG. 4 A.F.I.P. Acc. 525425. Tiny particles of bile are present in the cytoplasm of the giant liver cells. Bile is seen also in some of the Kupffer cells. Bile canaliculi are not visible. Kernechtrot. × 400.

LEGENDS FOR FIGURES

Jaundice of the neonatal period. Congenital anomaly of liver cells and bile canaliculi.

FIG. 1. Armed Forces Institute of Pathology accession no. 520738. The liver lobules are taken up by multinucleated giant liver cells; the portal areas are small and lightly infiltrated with lymphocytes and monocytes; the efferent veins are in proper position; the sinusoids are greatly narrowed. × 50.

FIG. 2. A.F.I.P. Acc. 520738. Multinucleated giant liver cells occupy the entire width of the columns. Their cytoplasm contains tiny particles of bile. Intercellular canaliculi are not evident. The sinusoids are narrow. Some of the Kupffer cells contain bile. × 360.

FIG. 3. A.F.I.P. Acc. 520738. Portal canal showing cross sections of immature appearing bile ducts with prominent lining cells, irregular lumina, and imperfect walls (D). The stroma of the portal field is moderately infiltrated with lymphocytes. The lymphatic channels (L) are greatly dilated. × 160.

FIG. 4 A.F.I.P. Acc. 525425. Tiny particles of bile are present in the cytoplasm of the giant liver cells. Bile is seen also in some of the Kupffer cells. Bile canaliculi are not visible. Kernechtrot. × 400.

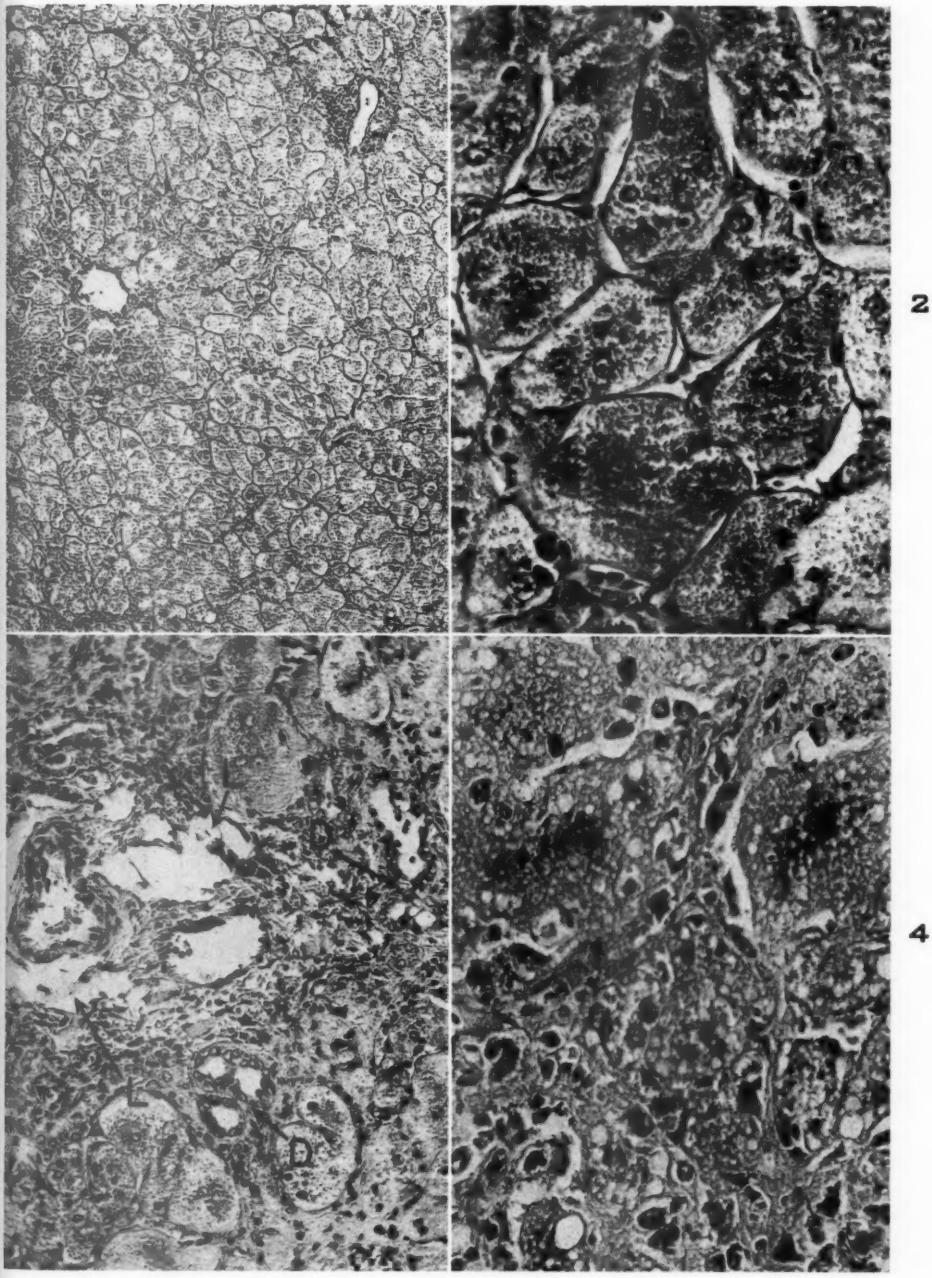
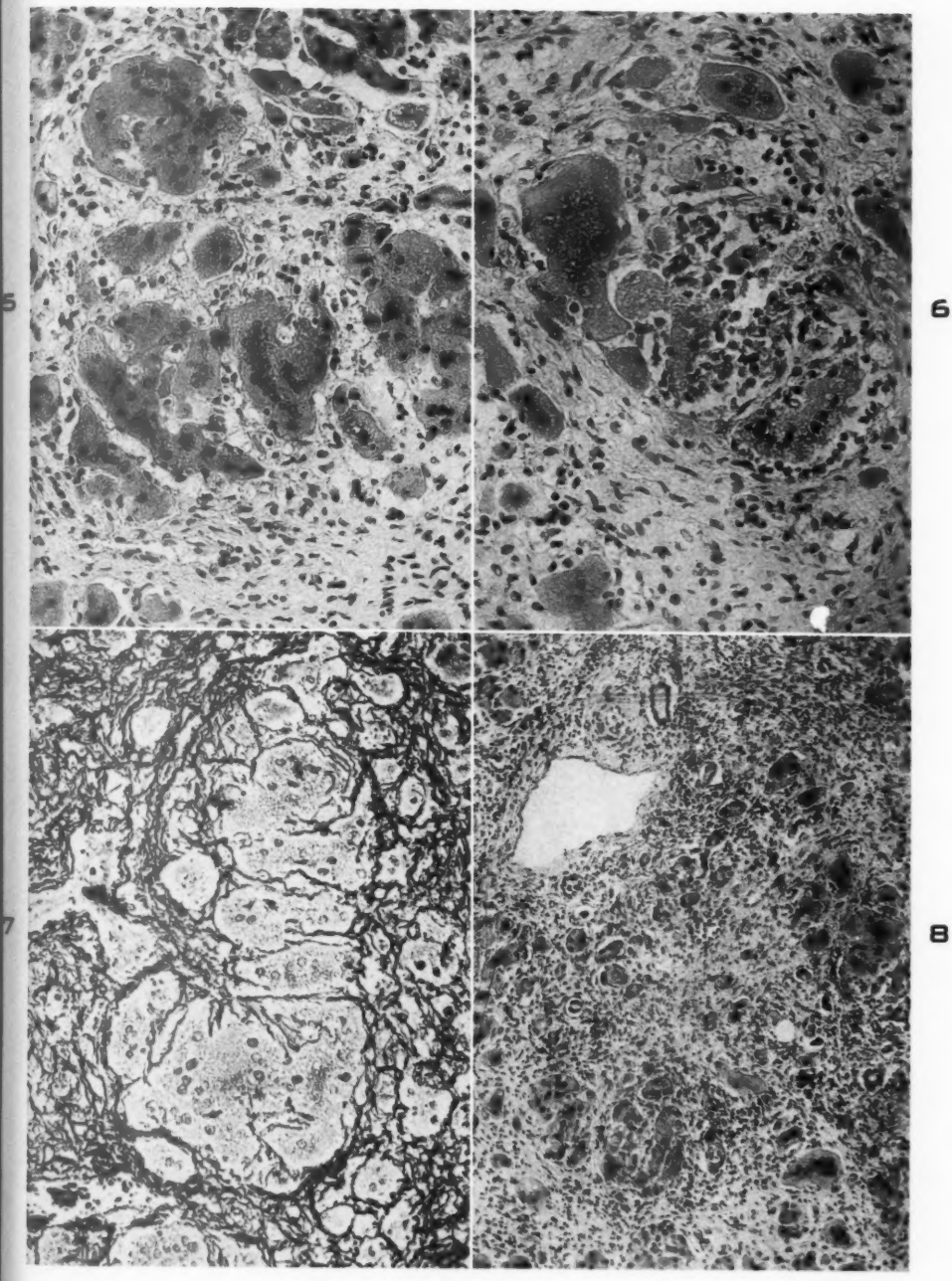

1
2
3
4

FIG. 5. A.F.I.P. Acc. 561123. Cirrhotic stage. Apparent increase in hepatic stroma with reduction in number and isolation of giant liver cells and disruption of lobular architecture. Tiny bile droplets are seen in the cytoplasm of the giant cells. No bile canaliculi are seen. × 185.

FIG. 6. A.F.I.P. Acc. 561123. Cirrhotic stage. Disintegrating giant liver cell with focal inflammatory reaction composed of monocytes, lymphocytes, and polymorphonuclear leukocytes. Apparent increase in hepatic stroma with isolation of giant cells and disorganization of lobular architecture. × 235.

FIG. 7. A.F.I.P. Acc. 525425. Cirrhotic stage. Isolated groups of giant liver cells embedded in collapsed and condensed reticulum fibers. Single giant cells are separated from others by the stroma. The sinusoids are quite narrow and sometimes appear obliterated. Wilder's stain. × 185.

FIG. 8. A.F.I.P. Acc. 561123. Cirrhotic stage. Diffuse fibrosis of liver with isolation of groups of giant liver cells and disorganization of lobular architecture. These groups are not regenerating islands but represent remains of columns of the giant liver cells. × 75.



LESIONS OF THE INTRAHEPATIC PORTAL RADICLES IN MANSON'S SCHISTOSOMIASIS *

FRANCISCO LICHTENBERG, M.D.†

From the Hospital das Clinicas, Universidade de Bahia, Brazil, South America, and the School of Medicine-School of Tropical Medicine, University of Puerto Rico, San Juan, P.R.

The adult worms of *Schistosoma mansoni* dwell in veins of the portal system, mostly those of the colon, and oviposition takes place in the corresponding venules in the wall of the colon. The organic lesions of schistosomiasis generally can be correlated with the vascular territory inhabited by the trematode. Faust[1] has shown this to be true also in the so-called ectopic lesions.

The vascular distribution of the eggs was strikingly demonstrated in one of our heavily infested cases (no. 125, Hospital das Clínicas). This patient was a 26-year-old man showing both advanced hepatic fibrosis and pulmonary arteritis. Eggs and pseudo-tubercles were found in eighteen different regions, including the central nervous system, myocardium, thyroid gland, and both kidneys (Figs. 1, 2, 3, and 4). Eggs and egg shells were demonstrated in renal arterioles; one of them in a glomerular hilus, presumably in an afferent vessel (Fig. 4). It was concluded that ova had been shunted through pathologic vascular anastomoses in the lungs, into the greater circulation, so that they were disseminated as emboli.

While the vascular distribution of ova is a well known feature of the disease, the lesions produced in blood vessels by *S. mansoni* have received little attention. The route taken by the egg from the vascular to the intestinal lumina during colonic oviposition is largely unknown.

Kohlschütter and Koppisch[2] have described a process of vascular extrusion characterized by adhesion of the egg to the vascular wall, coverage by endothelial cells which isolate the foreign body, and finally inflammatory change around the egg resulting in compression or obliteration of the involved vessel. At the intestinal venous and capillary levels, both gross and microscopic changes have been mentioned frequently and have been summarized recently by Valladares.[3] Inflammation of the larger portal trunks and of the main stem was mentioned many years ago by Letulle.[4] Koppisch[5] found several cases of

* Received for publication, November 3, 1954.

Read by title at the Fifty-second Annual Meeting of the American Association of Pathologists and Bacteriologists, Houston, Texas, April 7, 8, and 9, 1955.

† Now at Department of Pathology, University of Puerto Rico School of Medicine, San Juan, P.R.

extrahepatic portal thrombosis in a large series of necropsies but he considered this complication to be related to portal hypertension rather than to direct inflammatory excitation by the parasite. In the experimentally infested rabbit, Koppisch[6] described an early portal endophlebitis, characteristically "polypoid" in appearance. Review of the literature revealed only two descriptions of lesions affecting the intrahepatic portal radicles; namely, mention by Hashem[7] and by Bezerra Coutinho[8] of endophlebitic proliferation of vascular endothelium in radicles containing ova or remains of ova. In the lung, the arteritis described by Shaw and Ghareeb[9] is the best known and most destructive vascular lesion of schistosomiasis. Finally, in the case mentioned (no. 125, Hospital das Clínicas), inflammation of peripheral arterioles in the vicinity of impacted ova was observed (Fig. 5). Thus, while blood-vascular lesions have been observed in practically all localizations of schistosomiasis, their importance has not been emphasized sufficiently, particularly with reference to the liver.

Material and Methods

This study is based on 27 cases of Manson's schistosomiasis from a series of 52 consecutive non-selected necropsies at the Hospital das Clínicas of the University of Bahia (1952). These cases were studied from a clinicopathologic point of view and will be reported in detail elsewhere.[10] In addition, specimens of liver for biopsy had been obtained at laparotomy from 4 cases. The data obtained from the clinical and pathologic study were pooled, the gross material was reviewed, and a complete histologic study of the livers was undertaken, including the sectioning of new tissue blocks as necessary.

In addition to the routine hematoxylin and eosin, the following stains were used as needed: the Ziehl-Neelsen method for ova of *S. mansoni*,[11] the Unna-Tänzer orcein-methylene blue stain for elastic fibrils,[12] and Masson's trichrome stain for connective tissue. A highly satisfactory stain for vascular lesions of schistosomiasis is a combination of the first two stains: elastic fibers were stained overnight in orcein as in Unna-Tänzer's method[12] and ova were then stained by a modified Ziehl-Neelsen method,[11] followed by light counterstaining with methylene blue.

General Statistical Data

The 27 necropsy cases were divided into two main groups,[10] mild and severe, on the basis of anatomical lesions. The primary criterion was the presence or absence of intestinal fibrosis, hepatic fibrosis, or

pulmonary arteritis, and the secondary criterion was the presence or absence of evidences of portal or pulmonary hypertension. In this way, 20 cases (74.1 per cent) were found to be mild and 7 cases (25.9 per cent) were classified as severe. The latter usually showed a combination of advanced organic lesions of the intestine, liver, and lung, of which the hepatic changes proved to be the most constant. Six cases (22.2 per cent) had hepatic fibrosis, 3 of which (11.1 per cent) showed a typical Symmers' pipe-stem cirrhosis. Pulmonary arteritis was found in 5 cases (18.5 per cent), in 4 of which (14.8 per cent) it was accompanied by right ventricular hypertrophy. Three cases (11.1 per cent) had advanced fibrosis and rigidity of the colon.

These data show that the Brazilian cases of schistosomiasis were more severe and more heavily infested than those studied in Puerto Rico,[5] which is almost certainly due to a higher degree of infestation of the waters to which our cases were exposed and to more frequent reinfestation.[18]

The 4 biopsy specimens represented fragments of liver tissue obtained in the course of splenectomy. These cases had been diagnosed as Manson's schistosomiasis by parasitologic examination, and clinically they were classified as of the "splenomegalic form."

Vascular Lesions Observed

Lesions of the intrahepatic portal radicles of three types were observed: (1) substitution by granuloma, (2) sclerosis and narrowing, and (3) intrahepatic thrombophlebitis. Descriptions of these lesions follow.

Substitution by Granuloma (Figs. 6, 7, and 8). In the early stage of substitution by granuloma, the lumen of the portal radicle was still discernible, and it contained an egg or a more or less fragmented shell, more often than not engulfed by a multinucleated giant cell. The endothelium was swollen, proliferated, and partially desquamated. The media was partially or completely destroyed and invaded by leukocytes, epithelioid cells, macrophages, and numerous eosinophils (Figs. 6 and 7). Remains of elastic fibers could be demonstrated. As the lesion progressed, the vessel became completely occluded by a granuloma, and there was destruction of the muscular and elastic layers. Thus it was no longer possible to recognize the vascular origin of the lesion except by inference (Fig. 8). However, when the portal triads were inspected with special attention to the location of the vein, it was noticed that while some showed venous channels by-passing a granuloma, others did not seem to contain a vein at all. Serial sections

of two livers showed that the portal vein was indeed entirely substituted by a granulomatous formation.

Sclerosis and Narrowing (Figs. 9 and 10). The lesion of sclerosis and narrowing was found in portal fields showing enlargement, fibrosis, and chronic inflammation. When the portal triad was inspected, the vein at first seemed to be absent, but closer attention revealed a few compressed capillary channels surrounded by scattered cells with elongated nuclei and faintly eosinophilic cytoplasm. These structures could be identified as the remains of an almost atretic portal radicle and its fibrotic middle layer (Fig. 9). The identification was much easier with special techniques, especially with Masson's trichrome stain (Fig. 10). The lumen of the vessel was either narrowed or subdivided. The subintimal layer was greatly thickened and the fibrous tissue continued through the hiatuses between the muscle fibers into the dense connective tissue of the portal space outside of the vein. The muscle layer appeared atrophic and was separated into small fascicles. Usually there was little inflammatory change and the impression was that of a residual sclerosis rather than of an active process. While ova and granulomas might be present in the portal space affected, this was not the rule.

Intrahepatic Thrombophlebitis (Figs. 11, 12, 13, 14, and 15). Intrahepatic thrombophlebitis was the most severe vascular lesion found. It affected the larger veins in cases showing definite pipe-stem fibrosis. Since the fibrotic portal fields showed a highly irregular vascular pattern, it often was difficult to visualize the branch of the portal vein, but by special stains the lesion became evident (Figs. 11 and 12). The vein was only slightly smaller than normal, and its lumen was entirely substituted by fibrous and inflammatory tissue representing an organized thrombus. The tissue replacing the lumen might show tortuous, almost cavernous, vascular channels, which appeared to communicate with similar channels outside the vein through hiatuses in the muscle layer (Fig. 15). This tissue was infiltrated with lymphoid cells and occasional leukocytes. Numerous eggs could be demonstrated, occasionally surrounded by pseudo-tubercles. The media of the vessel was difficult to see with the hematoxylin and eosin stain, but could be visualized by special methods. Its muscle bundles were atrophied and separated by fibrous tissue and dilated capillary channels. The elastica was found to be partially destroyed and showed numerous duplications, hiatuses, and irregularities (Figs. 13 and 14). Groups of eggs could be seen between the hiatuses of the elastic layer (Fig. 14). All layers of the vessel wall appeared infiltrated with in-

flammatory cells which were found also in the surrounding portal fibrous tissue. The latter frequently presented eggs and pseudo-tubercles.

Intrahepatic thrombophlebitis could be recognized on gross examination of the cut surfaces of the liver. Systematic inspection of portal fields sometimes showed only two lumina corresponding to the hepatic artery and the bile duct, respectively, while the portal vein was replaced by a poorly defined, slightly porous, white tissue. This lesion was particularly well seen under the hand lens.

In addition to the lesions of the portal radicles, other vascular changes seen less constantly will be mentioned only briefly. These lesions appeared to be more frequent in the advanced cases and in those showing pipe-stem cirrhosis.

Capillary Telangiectasia of the Portal Fields (Fig. 15). In the most advanced manifestation of capillary telangiectasia of the portal fields, the dilated vascular channels had a distinctly "angiomatoid" aspect, paralleling the telangiectasia seen in the lung.[9] Grossly, the portal fields might be reddish and of a spongy appearance, instead of white and dense, as classically described.[5] The telangiectatic lesion was associated with the presence of ova, granulomas, and inflammatory foci.

Intrahepatic Arteriolar Sclerosis and Medial Hypertrophy (Fig. 10). Intrahepatic arteriolar sclerosis and medial hypertrophy were found in many advanced cases of schistosomiasis. Their degree and frequency appeared to be greater than in non-infested persons of similar age.

Incidence of Lesions

No vascular lesions were found in 8 necropsy cases (29.6 per cent), making 70.4 per cent the incidence of intrahepatic vascular lesions in the necropsy series. All 4 biopsies showed vascular lesions, but this figure is of little significance in view of the selected nature of the cases.

Substitution by granuloma, which represents the mildest change, was also by far the most frequent, having been found in all 19 necropsy cases showing vascular lesions. In many of these, the lesion was sporadic in distribution, and in only 9 cases (33.3 per cent) was it conspicuously frequent. It also was found in all biopsy cases. The lesion was seen both in mild and in severe cases, and with and without portal fibrosis; it did not show a significant correlation with portal hypertension. It seemed to be most conspicuous in 3 cases of rather recent, massive infestation, with numerous pseudo-tubercles of the liver.

Sclerosis and narrowing, and intrahepatic thrombophlebitis, on the other hand, usually were associated with hepatic fibrosis and with portal hypertension. Sclerosis and narrowing was found in 5 necropsy cases (18.5 per cent), 3 of which showed advanced hepatic fibrosis. The remaining 2 cases showed sporadic lesions, and were classified as mild, although one of them did show a slight degree of splenomegaly. It was found also in 3 of the 4 biopsy cases. The number of portal fields affected in the individual cases was somewhat variable, but in none was there any difficulty in finding representative lesions.

Intrahepatic thrombophlebitis was the most advanced lesion, as well as the rarest. It was found in only 3 necropsy cases (11.1 per cent), all considered severe, and showing pipe-stem cirrhosis and portal hypertension. As with the previous lesions, not all of the portal radicles were involved. In 2 of the cases only every fifth to sixth field appeared affected. In the remaining case, one third to one half of the rami were occluded.

Comment

The frequency of intrahepatic vascular lesions found in this study raises the question as to why they have not been emphasized by previous workers. One of the presumptive reasons for the lack of previous attention is the fact that the lesions described here leave the vessels either substituted by granulomatous tissue, or markedly atrophied and inconspicuous, so that it is necessary to search systematically for the portal radicles in order to discover these alterations which, furthermore, may be inapparent unless certain special stains are utilized.

A consistent theory about their pathogenesis cannot be offered at this time. The frequency of vascular lesions in other localizations of Manson's schistosomiasis has been discussed. It must be emphasized that the pulmonary arterial lesions are destructive and feature a rupture of the elastic membranes.[9,14] It seems significant that in the present study lesions of the elastic layer of the portal veins also have been found. However, the speed with which eggs become impacted and the vascular anatomy of the organs are different, and, while the lesions appear analogous, their pathogenesis may vary. It is conceivable that the embryo within the egg shell produces substances endowed with a histolytic effect in order to facilitate its extrusion and motion toward the site of elimination, but such substances have not been identified chemically to date. It seems of interest, however, that an acid-fast-staining substance was recently demonstrated in the eggs.[11] Oliver González[15] and others believe that the pathogenetic action of the schistosome is more intimately associated with the egg than with

other forms of the parasite. Lópes de Faría[16] has attributed great importance to the finding of hyaline thrombi and believes that an "allergic" mechanism is at work in the production of schistosomal pulmonary endarteritis. He also suggested that "substances produced by the ovum" are the cause of the endarteritis. Whether the mechanism of action of the eggs is predominantly toxic, lytic, of an allergic-immunologic nature, or mainly "mechanical," is a matter of speculation.

Of the lesions described here, both substitution by granuloma and intrahepatic thrombophlebitis appear to be due to an active inflammatory process associated with the presence of eggs. Fibrosis and narrowing of the portal venules is a late manifestation associated with portal fibrosis. It may represent either the scar of a previous phlebitic lesion or the result of fibrosis spreading from the outside to the inside of a vein in a previously inflamed portal field. Substitution by granuloma occurs early in the course of infestation, and probably does not lead to embarrassment of the portal circulation unless a large number of venules are involved. Fibrosis and narrowing are frequently accompanied by portal hypertension. Intrahepatic thrombophlebitis is found only in cases with Symmers' pipe-stem cirrhosis and is associated with portal hypertension. Anatomically, this lesion is analogous to pulmonary arteritis. Just as the latter represents the severest involvement of the lung, intrahepatic thrombophlebitis may signify the most severe lesion affecting the liver.

It must be emphasized that the thrombophlebitis, as described in this paper, is not the ordinary recanalization of a thrombus, but a true phlebitis, since all vessel layers are altered by inflammation. It is an intrahepatic process, not to be confused with extrahepatic portal thrombosis. Koppisch[5] has described the latter alteration in 3 of his 147 cases. In the series of 27 cases,[10] one showed thrombosis of the splenic vein which, however, did not coincide with intrahepatic thrombophlebitis. Furthermore, while extrahepatic portal thrombosis may complicate any hepatic disease producing portal hypertension, intrahepatic thrombophlebitis has been observed only in schistosomiasis.

There is little doubt that any form of hepatic fibrosis will eventually lead to intrahepatic vascular blocking and to portal hypertension,[17] but there are vast variations among different diseases of the liver in the degree of vascular participation. Portal hypertension is a predominant manifestation of severe Manson's schistosomiasis in all endemic areas.[5,18,19] The incidence and degree of splenomegaly seem to be higher than in other forms of hepatic fibrosis, and splenic Gandy-Gamna bodies, as well as intrasplenic hemorrhages, are particularly

frequent in this condition.[20] Phlebosclerosis also is frequent,[5] and thrombosis of the portal vein is not uncommon.[5] Esophageal varices are a prominent part of the clinical picture,[21] and recently it has been emphasized that they may exist in otherwise asymptomatic patients.[22] Portal hypertension of long duration, in the absence of liver cell failure, and resembling the Banti syndrome, is one of the characteristic forms of the disease.[19]

Attempts have been made to correlate the frequency of splenomegaly with toxic factors produced by the adult parasite.[20] However, this report shows that in Manson's schistosomiasis, the intrahepatic portal radicles are affected early in the course of the disease, even before fibrosis is fully developed, and that the later stages of the disease are accompanied by narrowing lesions of the portal veins.

It must be emphasized that these findings show only anatomical narrowing and do not permit judgment as to what degree of functional impairment should be attributed to it. Also, the ratio of obstructed to unobstructed vessels was not established. Injections of the hepatic vascular bed in the manner of McIndoe[23] may be helpful in clarifying these and other questions.

In spite of the reservations outlined, it is my distinct impression that the lesions of the intrahepatic portal vessels found in schistosomiasis are one of the causes of the high incidence of portal hypertension in this disease. In addition, the identification of these lesions establishes a parallel with other vascular lesions of schistosomiasis previously described in the intestine and in the lung, thus suggesting a somewhat more unified picture of their pathogenesis.

Summary and Conclusions

In a study of the intrahepatic portal radicles of 27 cases of Manson's schistosomiasis, hitherto undescribed vascular lesions were found in 19 (70.4 per cent).

The lesions were of three types: substitution by granuloma of a portal radicle; fibrosis and narrowing, a more advanced lesion found in fibrotic portal fields; and intrahepatic thrombophlebitis, an obstructive lesion of larger venous ramifications, which may be analogous to schistosomal pulmonary arteritis. Intrahepatic thrombophlebitis was found only in advanced cases showing pipe-stem cirrhosis.

Intrahepatic vascular lesions arise early in Manson's schistosomiasis, and are frequent in the severe cases. They may be one of the causes of the frequency and severity of portal hypertension in this disease.

The vascular lesions of Manson's schistosomiasis in the liver parallel the vascular lesions in other organs, most notably in the lung, and therefore suggest a similar pathogenesis.

I wish to thank Dr. Enrique Koppisch for his many valuable suggestions, and Mr. Antonio Puras for part of the photomicrographs reproduced here.

REFERENCES

1. Faust, E. C. An inquiry into the ectopic lesions in schistosomiasis. *Am. J. Trop. Med.*, 1948, **28**, 175–199.
2. Kohlschütter, E., and Koppisch, E. On the mode of extrusion of Schistosome ova from blood vessels into the tissues. *Schweiz. Ztschr. f. Path. u. Bakt.*, 1941, **4**, 357–369.
3. Valladares, C. Determinações intestinais na doença de Manson-Pirajá da Silva. Thesis: Fundação Gonzalo Moniz, Bahia, Brasil, 1953.
4. Letulle, M. Bilharziose intestinale. *Arch. de Parasitol.*, 1905, **9**, 329–439.
5. Koppisch, E. Studies on schistosomiasis mansoni in Puerto Rico. VI. Morbid anatomy of the disease as found in Puerto Ricans. *Puerto Rico J. Pub. Health & Trop. Med.*, 1940–41, **16**, 395–455.
6. Koppisch, E. Studies on schistosomiasis mansoni in Puerto Rico. IV. The pathological anatomy of experimental schistosomiasis mansoni in the rabbit and albino rat. *Puerto Rico J. Pub. Health & Trop. Med.*, 1937, **13**, 1–54.
7. Hashem, M. The aetiology and pathogenesis of the endemic form of hepatosplenomegaly "Egyptian splenomegaly." *J. Royal Egyptian M. A.*, 1947–48, **30**, 48–79.
8. Bezerra Coutinho, A. Aspectos histológicos das localizações viscerais da esquistosomose de Manson. *An. Fac. de med. do Recife*, 1934, **1**, 16–21.
9. Shaw, A. F. B., and Ghareeb, A. A. The pathogenesis of pulmonary schistosomiasis in Egypt with special reference to Ayerza's disease. *J. Path. & Bact.*, 1938, **46**, 401–424.
10. Lichtenberg, F.; Valladares, C., and Studart, J. Análise anatomoclínica de 27 casos de esquistossomose mansoni. To be published.
11. Lichtenberg, F., and Lindenberg, M. An alcohol acid-fast substance in eggs of *Schistosoma mansoni*. *Am. J. Trop. Med. & Hyg.*, 1954, **3**, 1066–1076.
12. Lillie, R. D. Histopathologic Technic. The Blakiston Co., Philadelphia, 1948, pp. 198–199.
13. Coutinho, J. O. Contribuição ao estudo da esquistossomose mansônica no estado da Bahia-Brasil. *Arq. de hig. e saúde pub.*, 1951, **16**, 3–42.
14. Andréa, J. Localizações pulmonares da doença de Manson-Pirajá da Silva. Thesis: Era Nova, Bahia, Brasil, 1949.
15. Oliver González, J. Personal communication.
16. Lópes de Faría, J. Histopatología da endarterite pulmonar esquistossomótica. Thesis: Empresa Gráfica da "Revista das Tribunais" Ltda., Sao Paulo, Brasil, 1952. *See also:* Cor pulmonale in Manson's schistosomiasis. I. Frequency in necropsy material; pulmonary vascular changes caused by schistosome ova. *Am. J. Path.*, 1954, **30**, 167–193.
17. Himsworth, H. P. The Liver and Its Diseases. Harvard University Press, Cambridge, Mass., 1948, p. 121.

18. Day, H. B. Bilharzial cirrhosis. (Egyptian splenomegaly.) *J. Trop. Med.*, 1933, 36, 17–23.

19. Rodríguez da Silva, J. Estudo clinico da esquistossomose mansoni (doença de Manson-Pirajá da Silva). Thesis: Grafica Laemmert Ltda., Río de Janeiro, Brasil, 1949.

20. Meira, J. A. Esquistosomiase mansoni hepato-esplénica. Thesis: Tipografía Edanec Ltda., Sao Paulo, Brasil, 1951.

21. Muñoz-MacCormick, C. E. The injection treatment of esophageal varices due to Manson's schistosomiasis. Preliminary report. *Laryngoscope*, 1948, 58, 992–1012.

22. Palmer, E. D., and Jahnke, E. J., Jr. Observations on portal hypertension among schistosomiasis patients with relatively insignificant complaints. *Am. J. Trop. Med. & Hyg.*, 1954, 3, 139–146.

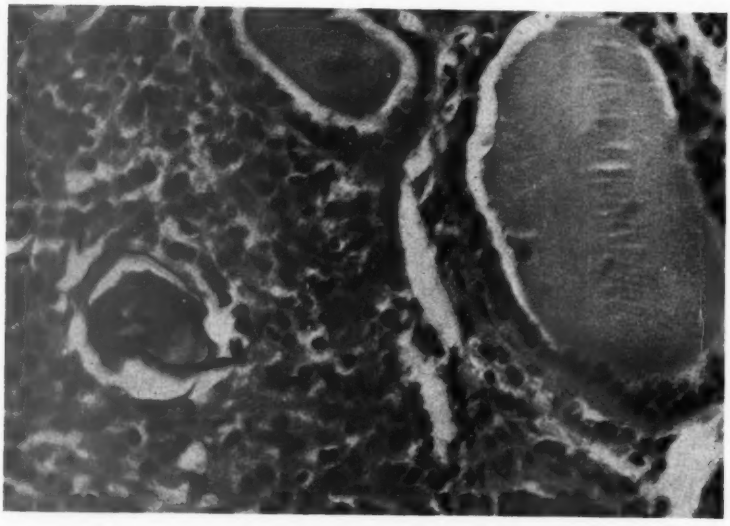
23. McIndoe, A. H. Vascular lesions of portal cirrhosis. *Arch. Path.*, 1928, 5, 23–42.

LEGENDS FOR FIGURES

FIG. 1. Schistosome egg in thyroid gland, surrounded by a pseudo-tubercle. Hematoxylin and eosin stain. × 400.

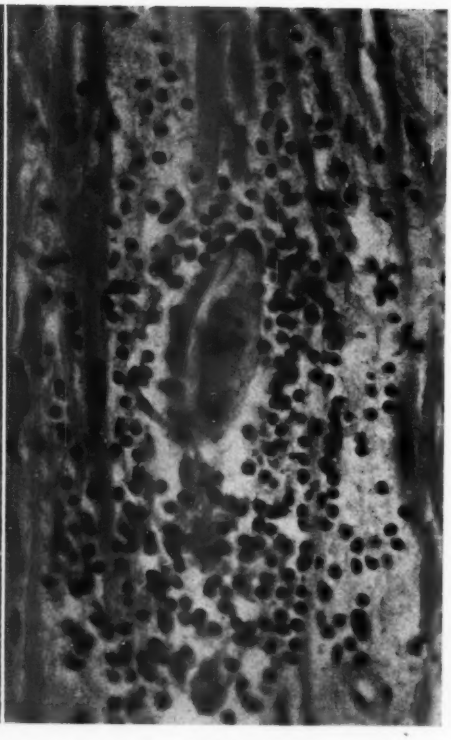
FIG. 2. Egg in white substance of cerebellum, near a capillary containing a hyaline thrombus; the surrounding glial fibers have a reticulated appearance. Hematoxylin and eosin stain. × 400.

FIG. 3. Egg in the myocardium. The nuclei of the embryo are seen through the tangentially sectioned egg shell. The surrounding cells are mainly lymphocytes. To the left of the egg in the area of inflammation there is a partially lined space suggesting a capillary. Hematoxylin and eosin stain. × 400.

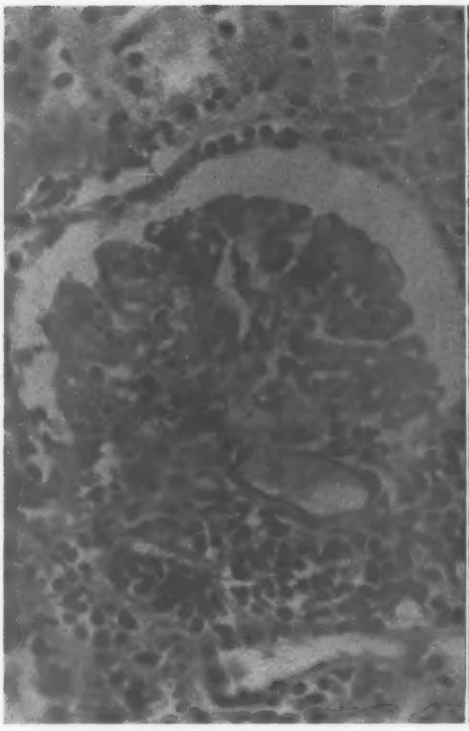


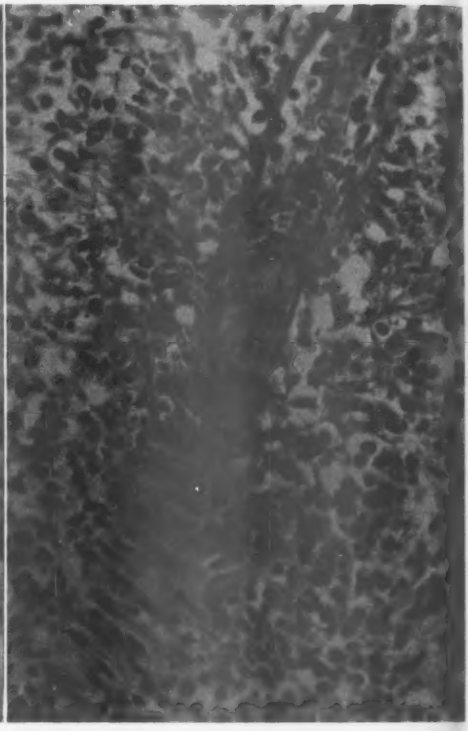
1

2



3





4 5

FIG. 4. Egg shell impacted in a vessel of the glomerular hilus, presumably an afferent arteriole, surrounded by inflammatory cells. The black granules are produced by formalin pigment. Hematoxylin and eosin stain. × 400.

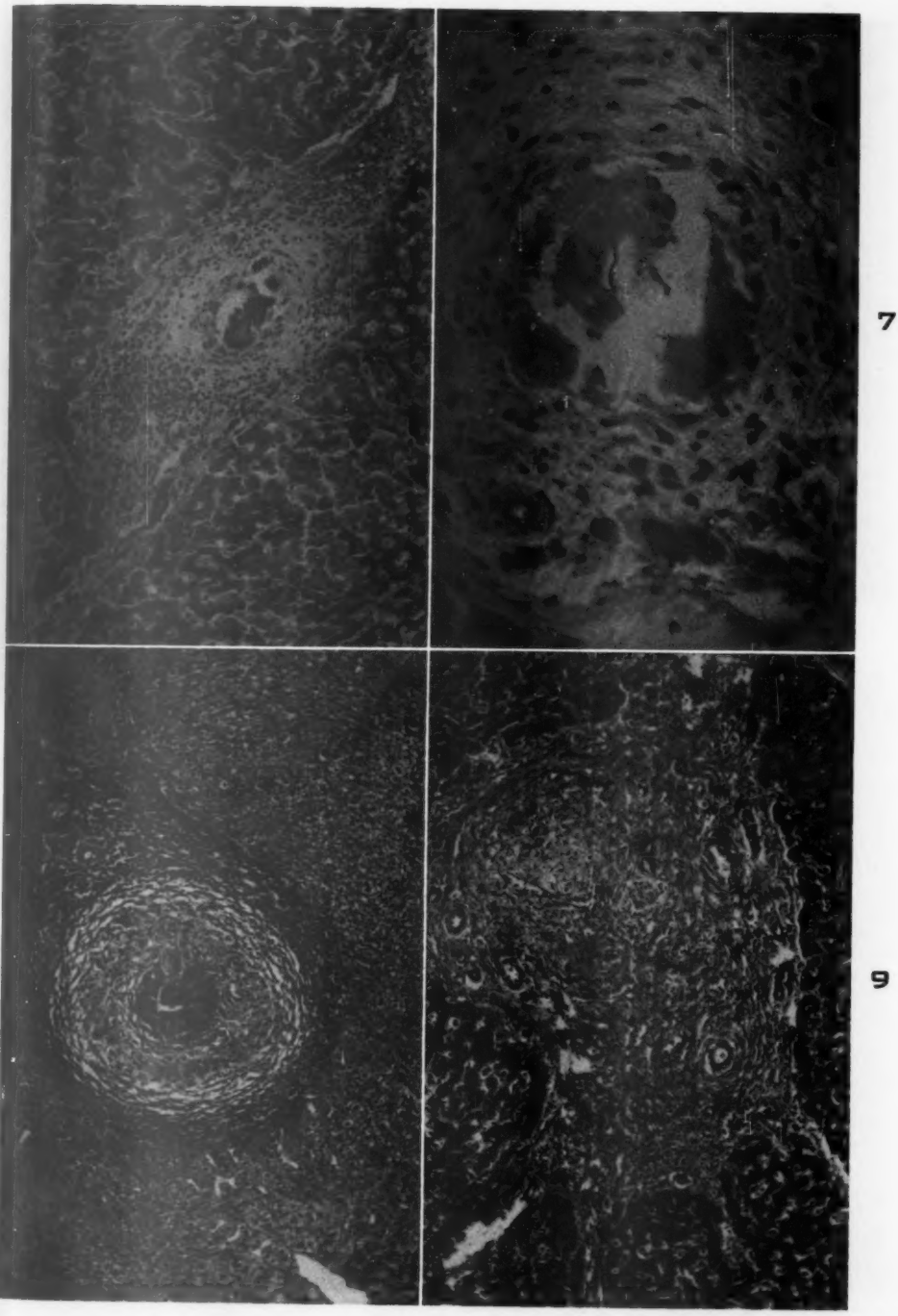
FIG. 5. Arteriole of renal cortex with impacted egg shell sectioned tangentially. Fibrinoid material is present below and to the right. The vessel is surrounded by a dense halo of inflammatory cells. Hematoxylin and eosin stain. × 400.

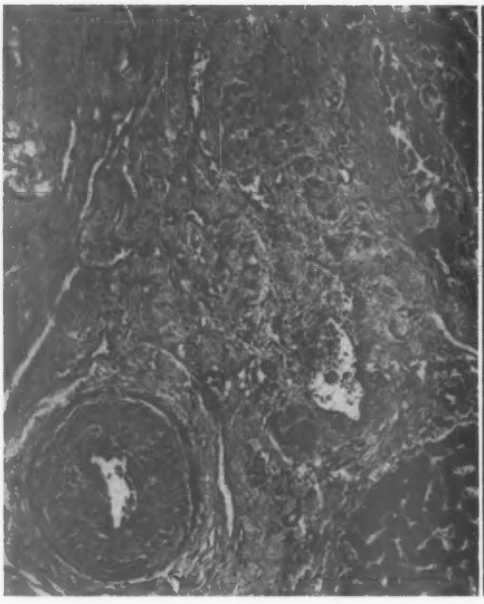
FIG. 6. Portal space showing initial phase of substitution by granuloma. Arteriole and bile capillary are below at the left. The lumen of the portal vein is occupied by an egg shell engulfed by giant cells. The proliferating endothelium resembles epithelioid cells. The muscle layer is not recognizable. There is marked round cell infiltration. Hematoxylin and eosin stain. × 120.

FIG. 7. The small arteriole (lower left) shows its elastica interna. Remains of elastic fibers are seen concentrically around the inflamed vein. Proliferation of endothelium around the egg is not yet sufficient to obliterate the vessel completely. Orcein-methylene blue stain. × 400.

FIG. 8. Complete substitution by granuloma. Arteriole and bile duct are in the upper left. The venule is substituted by a well developed pseudo-tubercle with centrally placed egg. Hematoxylin and eosin stain. × 80.

FIG. 9. Chronically inflamed and enlarged portal field showing sclerosis and narrowing of portal vein. Several small bile ducts and arterioles can be observed, but the main ramus of the portal vein is not immediately visible. The slit-like opening extending to the left from the center represents the remains of its lumen. About it there are occasional groups of elongated, dark staining cells representing the remains of the muscle layer. Masson's trichrome stain. × 80.





10

11

FIG. 10. Part of an enlarged portal field. The artery shows marked intimal sclerosis and medial hypertrophy. The fibrous tissue is dense and contains numerous branching capillaries. The former outlines of the vein are marked by scattered atrophic muscle bundles, roughly elliptic (to the right of the center). Subdivision of the lumen and connection with adventitial capillaries may be noted; also absence of inflammatory infiltration. Masson's trichrome stain. × 120.

FIG. 11. Low-power view of large portal field in a case of advanced pipe-stem cirrhosis. This area is adjacent to the field seen in Figure 12 after elastica and acid-fast staining. It represents a partially obliterated portal vein with intrahepatic thrombophlebitis. The outline of the vessel is defined with difficulty. Hematoxylin and eosin stain. × 35.

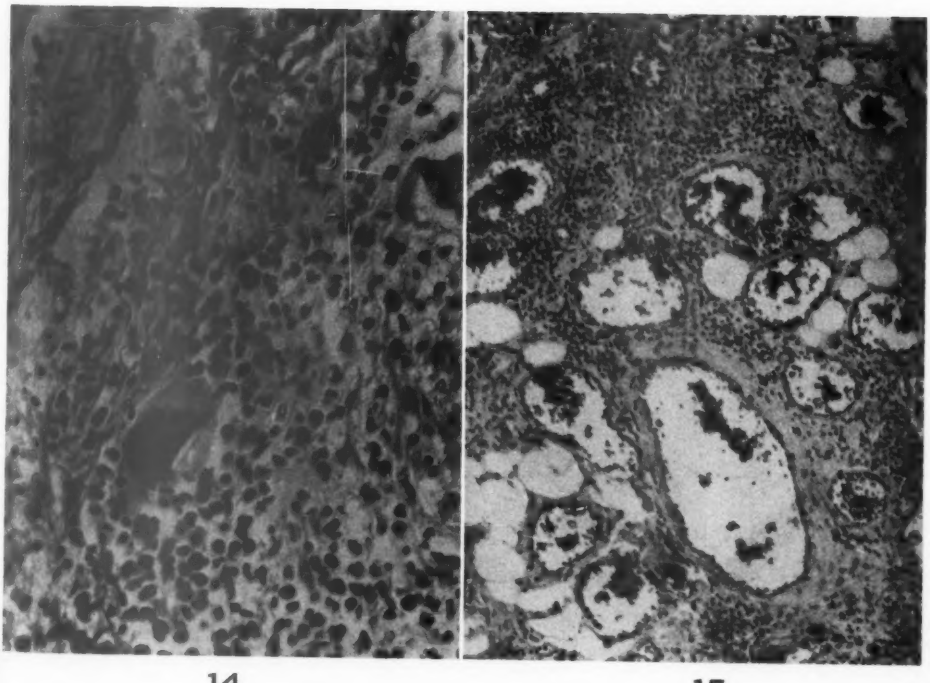
FIG. 12. Low-power view of obliterated and inflamed vein showing intrahepatic thrombophlebitis. There are irregularities and interruptions of elastica, and dense inflammatory infiltration of all layers. Orcein-Ziehl-Neelsen's and methylene blue stains. × 35.

FIG. 13. Greater magnification of a field similar to that seen in Figure 12, showing irregularities of elastic layer. Orcein-Ziehl-Neelsen's and methylene blue stains. × 80.

FIG. 14. Detail of eggs close to the elastic fibers, which appear irregular and partially interrupted. The inflammatory elements are mostly histioid, lymphoid, and plasma cells. One egg shell is engulfed by a multinucleated giant cell. Orcein-Ziehl-Neelsen's and methylene blue stains. × 365.

FIG. 15. Telangiectasia of newly formed capillaries of a large portal field near the hilus of the liver, resembling "angiomatoids" of lung. A few fat cells are present and the inflammatory infiltration is dense and mainly lymphoid. Hematoxylin and eosin stain. × 90.

12 13



14 15

HEMANGIOPERICYTOMA IN THE DOG *

R. M. MULLIGAN, M.D.

From the Department of Pathology, University of Colorado, School of Medicine, Denver 20, Colo.

In 1948, a study of certain spindle cell tumors of the dog by hematoxylin and eosin stain alone resulted in an apparently valid classification,[1] although in several instances diagnosis was somewhat uncertain. In 1953 and 1954, 182 spindle cell tumors of dogs were classified by hematoxylin and eosin, Wilder's reticulum,[2] and Gomori's trichrome[3] stains with much more satisfying and conclusive results than formerly. The most striking findings concerned 68 tumors with the distribution and histologic features described by Stout and co-workers[4-6] for hemangiopericytoma. The purposes of this paper are to describe these 68 cases of hemangiopericytoma in the dog and to discuss the differential histologic diagnosis with respect to tumors with spindle cells of three other types; namely, fibroblasts, leiomyoblasts, and neurilemoblasts (closely applied nerve sheath cells). No instance of encapsulated neurilemoma[7] in the dog has yet been studied in this laboratory.

MATERIALS AND METHODS

Most of the tumors were obtained from veterinarians in the Denver and Los Angeles metropolitan areas, with some from other localities in the United States. The names of the donors have been listed previously.[1] The primary tumor was obtained surgically in 44 cases; the primary tumor, at necropsy in 11; the primary tumor and its recurrence (first in 4; first, second, and third in 1), surgically in 5; the recurrence (first in 4, fourth in 1) surgically in 5; and the first recurrence, at necropsy in 3. The tumors were fixed in 4 per cent aqueous formaldehyde, imbedded in paraffin by the dioxane technique, and cut 6 to 8 μ thick. The following staining techniques were used for the blocks of some or all of the 75 specimens available for study: hematoxylin and eosin, Wilder's reticulum stain counterstained by 0.5 per cent aqueous aniline blue or by the van Gieson method, and Gomori's trichrome stain. Technical difficulties were encountered in 8 instances with the Wilder reticulum stain.

* Received for publication, December 11, 1954.

Observations

Sex and Age. As to sex, the 68 dogs were 33 intact males, 2 castrated males, 28 intact females, and 5 spayed females. The age distribution was: 2 to 5 years, 3 dogs; 6 to 10 years, 23; 11 to 15 years, 36; and 16 to 20 years, 6.

Location. The sites of the 68 tumors were: posterior extremities, 20; anterior extremities, 18; abdomen, 12; thorax, 9; head, 6; neck, 1, and not specified, 2. All were solitary and subcutaneous, except 2 listed for the abdomen. One of these was in the jejunum and had produced melena; the other was posterior to the uterine cervix. The great majority were located in the extremities (56 per cent) or trunk (31 per cent).

Breed. The following breeds were represented: fox terrier, 13; mongrel shepherd, 13; mongrel terrier, 7; mongrel, 5; cocker spaniel, 4; Boston terrier, 3; German shepherd, 3; Scotch terrier, 2; Irish setter, 2; collie, 2; mongrel spaniel, 2; and Chesapeake Bay retriever, Samoyede, Chihuahua, poodle, whippet, great Dane, old English sheepdog, Irish terrier, dachshund, boxer, Doberman pinscher, and chow chow, 1 each.

Gross Findings. The tumors were firm and lobulated; and gray, gray-yellow, white, or pink-gray and red stippled, streaked or mottled. The size of the primary tumor at the time of removal was given in cubic centimeters and grams in 37 cases, in cubic centimeters only in 15, and in grams only in 1 case. The volume of 52 tumors was 10 cc. or less in 13 cases, 11 to 100 cc. in 22, 101 to 500 cc. in 13, 501 to 1000 cc. in 3, and 1400 cc. in 1 case. The weight of 38 tumors was 10 gm. or less in 4, 11 to 100 gm. in 23, 101 to 500 gm. in 9, and 501 to 1000 gm. in 2 cases. The duration of the primary tumor, as stated in 33 cases, was 1 month to 6 years. The larger examples had been present for 2 years or more.

Recurrence and Metastasis. Recurrence was found in 13 cases. The recurrent tumors developed within 5 to 28 months after excision of the primary growth in 12 instances, with 1 recurrence in 10 cases, 3 in 1, and 4 in 1. In the 13th case, a "few" recurrences were observed in 4 years. Metastasis was not noted in any of the 68 cases.

Microscopic Examination

The constant association of neoplastic capillaries and pericytes was the most important characteristic for the neoplasms of this group (Figs. 1 to 20). The relationship of capillaries lined by endothelial cells to the laminated pericytes around them was so intimate as to

suggest that their survival was interdependent. A like relationship of capillaries to fibroblasts, smooth muscle cells, or nerve sheath cells was not seen in tumors composed of these cells. Rather, neoplastic cells of these three types seemed to tolerate, but not to have close association with, the nourishing blood vessels, capillary or otherwise. In the neoplasms considered hemangiopericytomas, the capillaries might be open and obvious or collapsed and occult. The capillaries were lined by a single layer of ordinary endothelial cells. In some neoplasms they were set closely together and were cuffed by slender strands of pericytes; in others they were disposed farther apart and were surrounded by fat bundles of pericytes. When packed around relatively closely set capillaries, the pericytes were short and spindle-shaped in comparison to fibroblasts, but had slender, tapered processes and oblong or ovoid nuclei. When loosely arranged between more widely spaced capillaries, the pericytes varied from short spindle-shaped to polyhedral elements and contained ovoid or rounded nuclei, which sometimes appeared as if floating in pools of cytoplasm (Fig. 11). The oblong, ovoid, or rounded nuclei had definite membranes, small or medium-sized distinct nucleoli, and finely meshed, loose chromatin with densely stippled dots on the chromatin threads. Mitotic figures varied in number and were not especially abnormal in primary or recurrent tumors. The pericytes sometimes were spread apart by pale, acidophilic or basophilic fluid or were slenderized to tapered spindle shape by local increase of collagen fibrils among them. The nuclei of these flattened cells became oblong and the chromatin became condensed and coarsely granular. Large areas might undergo sclerosis by the extension of the collagen fibrils from the walls of the capillaries to obliterate their lumina and to choke the growth of adjacent pericytes (Fig. 20). These sclerotic areas were marked occasionally by calcium deposits. When small areas of the tumor were damaged, the cells tended to become rounded, loosened, and intermingled with histiocytes, foreign body giant cells, and lymphocytes. Sheets of lipid macrophages also appeared in damaged areas. Even in well preserved tumors, some capillaries might be cuffed by lymphocytes. Less frequently, large areas of tumor showed coagulation necrosis. In some tumors, medium and large atypical veins filled with blood or thrombi communicated with neoplastic capillaries, which radiated into surrounding neoplasm.

Silver impregnation of reticulum fibers in the walls of the capillaries often was necessary to highlight the capillaries against the encircling strands, bundles, and whorls of proliferated pericytes (Figs. 2, 4, 6, 8,

10, 12, 14, 16, and 18). The capillaries were surrounded by thin to thick collars of reticulum fibers which were blackened by silver in direct proportion to their bulk. Fine, short reticulum fibers were conspicuously branched between capillaries in some tumors (Figs. 6, 10, and 12), but were scarce in others (Figs. 2, 4, 8, 14, and 16). Heavy tangles of reticulum fibers appeared in sclerotic areas. The distribution of reticulum fibers was identical with that of collagen fibrils, since the black fibers of the reticulum stain coincided with the light green collagen fibrils of the trichrome stain in the walls of the capillaries, around them, and among the pericytes, which with the trichrome stain had pale green cytoplasm lacking fibrils.

When at a second operation, the first recurrence of a hemangiopericytoma was excised, the vascular pattern of the peripheral remnant of the inadequately excised primary tumor was retained, but the vascular pattern of the central recurrent tumor was lost (Figs. 15 and 16). Solid sheets of pericytes were interposed among scattered capillaries in nondescript fashion. Relatively short, fine to medium coarse reticulum fibers encircled pericytes disposed singly or in small clusters.

Comment

Although hemangiopericytoma in the dog has been recognized in the Veterinary Pathology Section of the Armed Forces Institute of Pathology[8] since 1946, the only example found in the literature was detailed by Stout[4] in 1949 for case 25 of his series. The subject was a female collie, 7 years old, with an encapsulated tumor of an elbow, 70 by 38 mm., for 7 months.

Hemangiopericytoma may be wrongly classified as other types of spindle cell tumor as indicated by Stout[4] when he stated: "It occurred to the writer that the rounded and spindle-shaped cells of these peculiar tumors, which had hitherto apparently been called vascular fibrosarcoma or passed unnoticed, might also be derivatives of the pericyte, and without any real scientific basis in support, the tumor was christened 'hemangiopericytoma.' " This became obvious in a check of the illustrations for Chapter IV of *Neoplasms of the Dog*,[1] since A and B of Plate XXXI, C of Plate XXXII, and A of Plate XXXIII, designated fibrosarcoma and A and C of Plate XXXIV, B and C of Plate XXXV, and B of Plate XXXVI diagnosed nerve sheath tumors are actually hemangiopericytomas. The chastening effect of the discovery of these errors has been somewhat counterbalanced by the attainment of much greater accuracy in the diagnosis of the bulk of spindle cell tumors than formerly. An irreducible minimum of such tumors in

man, in the dog, and in other species must remain undiagnosed, even though adequate tissue is available for study.

Hemangiopericytoma accounted for 8.8 per cent of the 773 tumors of mesenchymal (748) and of nerve sheath cell origin (25) in the dogs studied in this laboratory. This may be compared with 74 benign hemangiopericytomas among 7,337 mesenchymal tumors, or 1.0 per cent, and 32 malignant hemangiopericytomas among 1,349 malignant mesenchymal tumors, or 2.4 per cent, observed in man by Stout.[9]

As in man, hemangiopericytoma of the dog occurs with about equal sex distribution, but is more frequent in dogs 6 years of age (the beginning of the "cancer age"[1]) and older as contrasted with an appearance at any age in man.[4] The location is preponderantly subcutaneous, with extremities and trunk often affected in both species. No breed of dog is especially predisposed. Although the fox terrier accounted for 13 tumors, or 20 per cent, and the mongrel shepherd for a like number of the 68 described, the high frequency of these two breeds in the dog population of Denver[10] in 1947 makes their apparent predisposition to hemangiopericytoma of doubtful significance. Racial predisposition for this tumor in man is uncertain. The duration, gross appearance, and microscopic characteristics are similar in man and the dog. In neither species can hemangiopericytoma be called benign or malignant by histologic appearance alone. Recurrence of some tumors is observed for each species, but metastasis was not found in any of the 68 cases in the dog. Possibly, further follow-up of the 44 dogs from which surgical specimens only were obtained might yield instances of metastasis. The loss of vascular pattern in recurrent hemangiopericytoma in the dog is a noteworthy finding which, to my knowledge, has not been described for this tumor when recurrent in man.

The spindle cell tumors of the dog studied for differential diagnosis from hemangiopericytoma with the three stains indicated were 25 fibromas, 7 fibrosarcomas, 39 leiomyomas, 18 leiomyosarcomas, 19 diffuse neurilemomas, and 6 malignant neurilemomas. The characteristics of the reticulum fibers and the cytoplasmic reaction of the tumor cells in these six types of spindle cell neoplasms are summarized briefly.

In fibroma, the reticulum fibers are long, thick, coarse, wiry, have broad, irregular waves, and are disposed among cells. In fibrosarcoma, they are of coarse to medium caliber, are finely barbed, and pass between cells or are often wrapped around single cells or small clusters of cells. In leiomyoma and leiomyosarcoma, the reticulum fibers are

coarse to medium, wiry or finely wavy, usually long, disposed among cells, often split longitudinally, and frequently they are marked by fine, short, lateral branches oriented more or less perpendicularly to the long axis of the cells. In diffuse neurilemoma, compact areas show coarse to fine, long to short, wiry or irregularly wavy reticulum fibers often split lengthwise and disposed among cells. In edematous areas conspicuous in this type of tumor, the fibers are short, coarse to fine, irregularly wavy, often split lengthwise, and disposed among cells. In malignant neurilemoma, the reticulum fibers are coarse to medium, have broad, irregular waves, are long, are often split lengthwise, and are disposed among cells.

With the Gomori trichrome method, leiomyoma and leiomyosarcoma usually give positive tinging of the fibrils within the cells. These fibrils appear as fine red filaments in longitudinal section and as minute dots in cross section. The cell processes of fibroma, fibrosarcoma, diffuse neurilemoma, and malignant neurilemoma stain light to medium green with this method.

In none of the spindle cell tumors of the other six types did reticulum or trichrome stains demonstrate a relationship of capillaries to the spindle cells like that seen for pericytes in hemangiopericytoma.

Summary

Of 68 hemangiopericytomas in dogs, 35 occurred in males and 33 in females; 65 were noted in dogs 6 years of age and older. All 68 tumors were solitary and frequently lobulated; 66 were subcutaneous; 59 were located on the extremities or trunk. No predilection for breed was recognized. Recurrence was observed in 13 cases, but metastasis was not seen in any case. The constant association of neoplastic capillaries and pericytes is most characteristic for hemangiopericytoma, in which the relationship of capillaries lined by endothelial cells to pericytes laminated around them is so intimate as to suggest that their survival is interdependent. A like relationship of capillaries to fibroblasts, smooth muscle cells, or nerve sheath cells is not seen in tumors derived from these cells, histologic differentiation of which from hemangiopericytoma can be made in most instances by reticulum and trichrome stains.

REFERENCES

1. Mulligan, R. M. Neoplasms of the Dog. Williams & Wilkins Co., Baltimore, 1949, 135 pp.
2. Wilder, H. C. An improved technique for silver impregnation of reticulum fibers. *Am. J. Path.*, 1935, 11, 817–819.

3. Gomori, G. A rapid one-step trichrome stain. *Am. J. Clin. Path.*, 1950, **20**, 661–664.

4. Stout, A. P. Hemangiopericytoma. A study of twenty-five new cases. *Cancer*, 1949, **2**, 1027–1054.

5. Stout, A. P., and Murray, M. R. Hemangiopericytoma; a vascular tumor featuring Zimmermann's pericytes. *Ann. Surg.*, 1942, **116**, 26–33.

6. Stout, A. P., and Cassel, C. Hemangiopericytoma of the omentum. *Surgery*, 1943, **13**, 578–581.

7. Stout, A. P. Tumors of the Peripheral Nervous System. Atlas of Tumor Pathology, Section II, Fascicle 6, Subcommittee on Oncology of the Committee on Pathology of the National Research Council. Armed Forces Institute of Pathology, Washington, D.C., 1949, 57 pp.

8. Jones, T. C. Personal communication.

9. Stout, A. P. Tumors of the Soft Tissues. Atlas of Tumor Pathology, Section II, Fascicle 5, Subcommittee on Oncology of the Committee on Pathology of the National Research Council. Armed Forces Institute of Pathology, Washington, D.C., 1953, 138 pp.

10. Mulligan, R. M. A statistical survey of the breeds of dogs licensed in Denver in 1947. *North. Am. Vet.*, 1949, **30**, 445–446.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Hemangiopericytoma, 52 cc., 48 gm.; a primary subcutaneous, abdominal tumor of a male bull terrier, 11 years old. Hematoxylin and eosin stain. × 330.

FIG. 2. Same tumor as in Figure 1. Wilder's reticulum stain counterstained with aqueous aniline blue. × 330.

FIG. 3. Hemangiopericytoma, 90 cc., 41 gm.; a primary subcutaneous tumor of the right foreleg of a castrate male old English sheepdog, 17 years old. No recurrence 8 months later. Hematoxylin and eosin stain. × 330.

FIG. 4. Same tumor as in Figure 3. Wilder's reticulum stain counterstained with aqueous aniline blue. × 330.

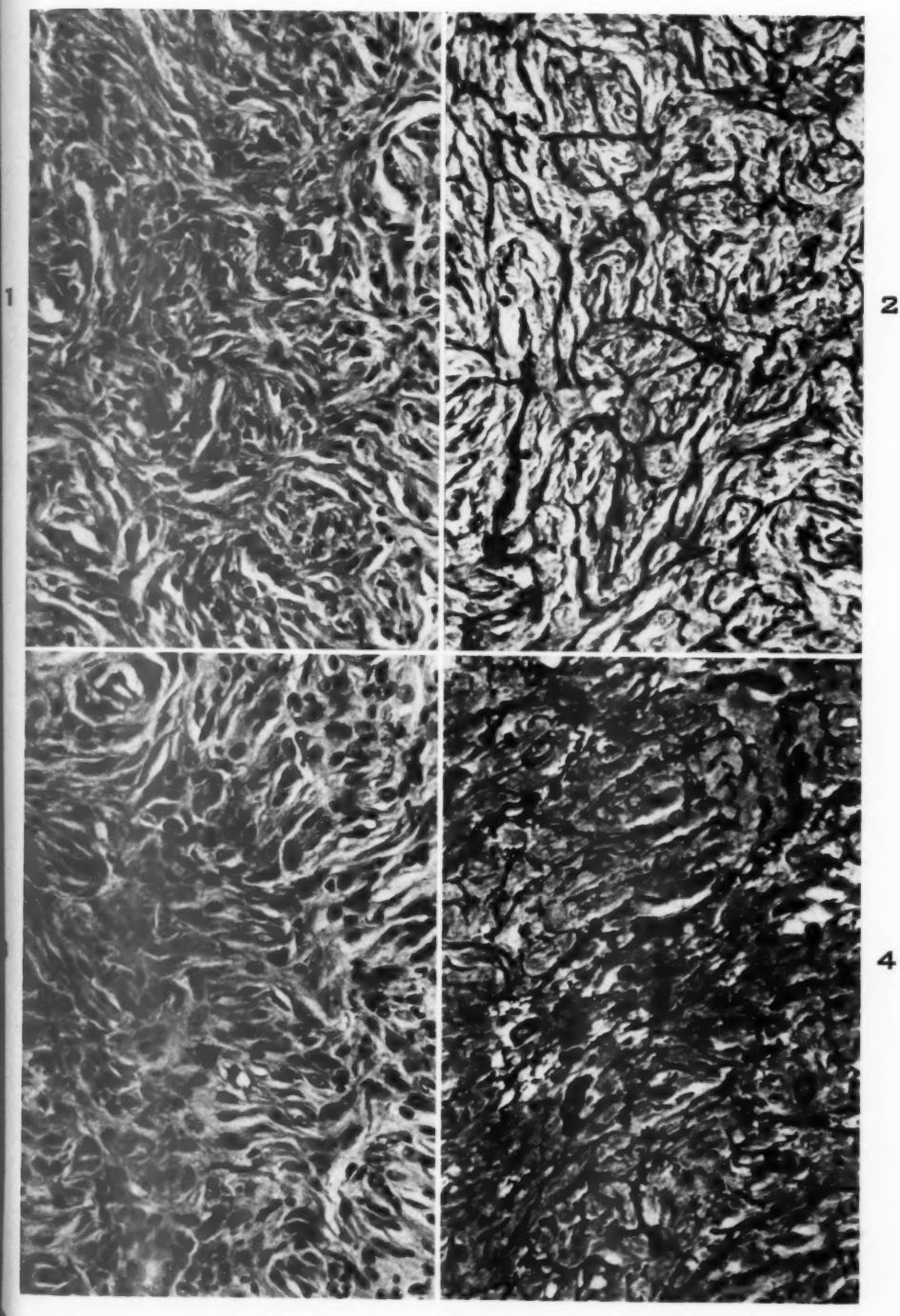


FIG. 5. Hemangiopericytoma, 30 cc., 15 gm.; a subcutaneous primary tumor of the left hind leg of a male fox terrier, 12 years old; had been noted for several months. Heavy collars of collagen fibrils around capillaries. Hematoxylin and eosin stain. × 330.

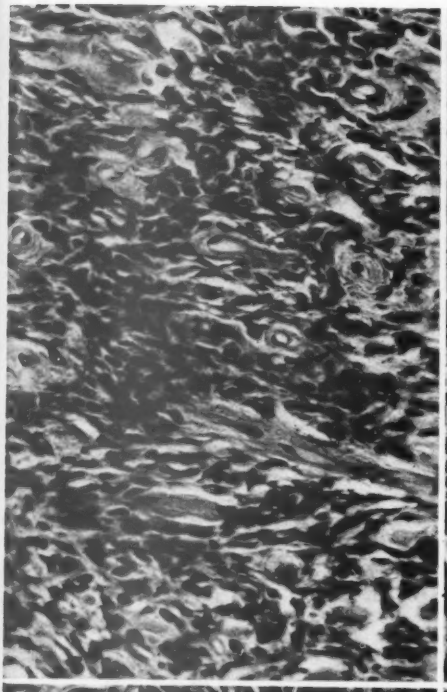
FIG. 6. Same tumor as in Figure 5. Wilder's reticulum stain counterstained with aniline blue. × 330.

FIG. 7. Hemangiopericytoma, "size of grapefruit"; a primary subcutaneous tumor of the ventral thoracic region, female mongrel terrier, 18 years old; had been noted for 2 years. Hematoxylin and eosin stain. × 330.

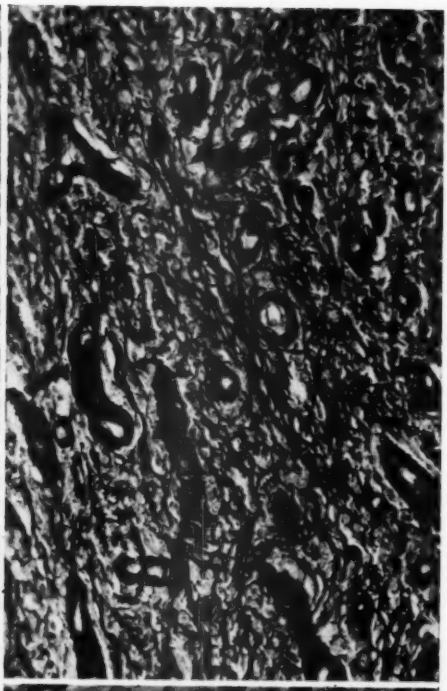
FIG. 8. Same tumor as in Figure 7. Wilder's reticulum stain counterstained by van Gieson's method. × 330.

5

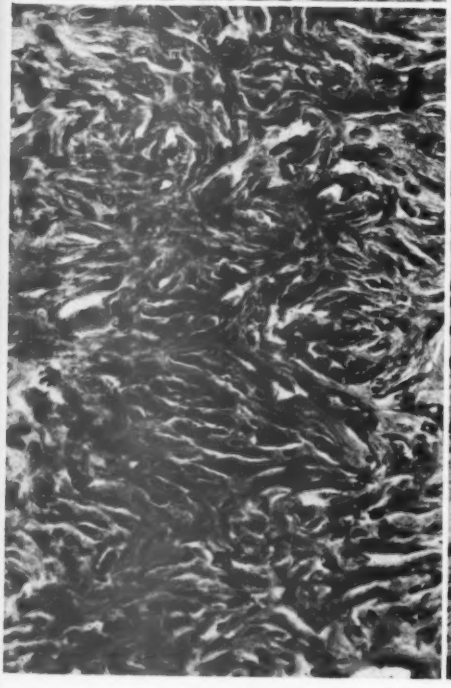
7



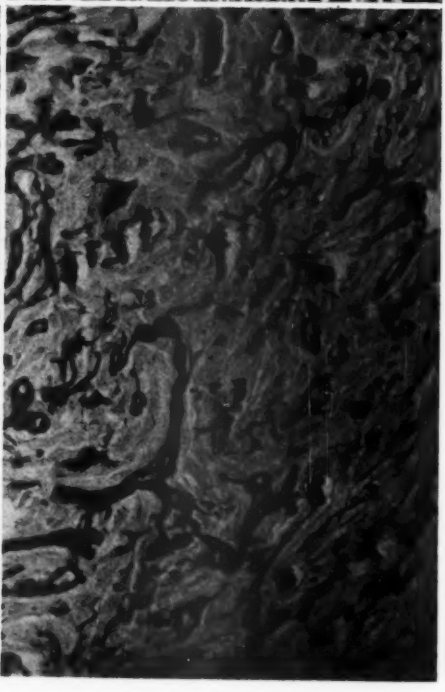
5



6



7



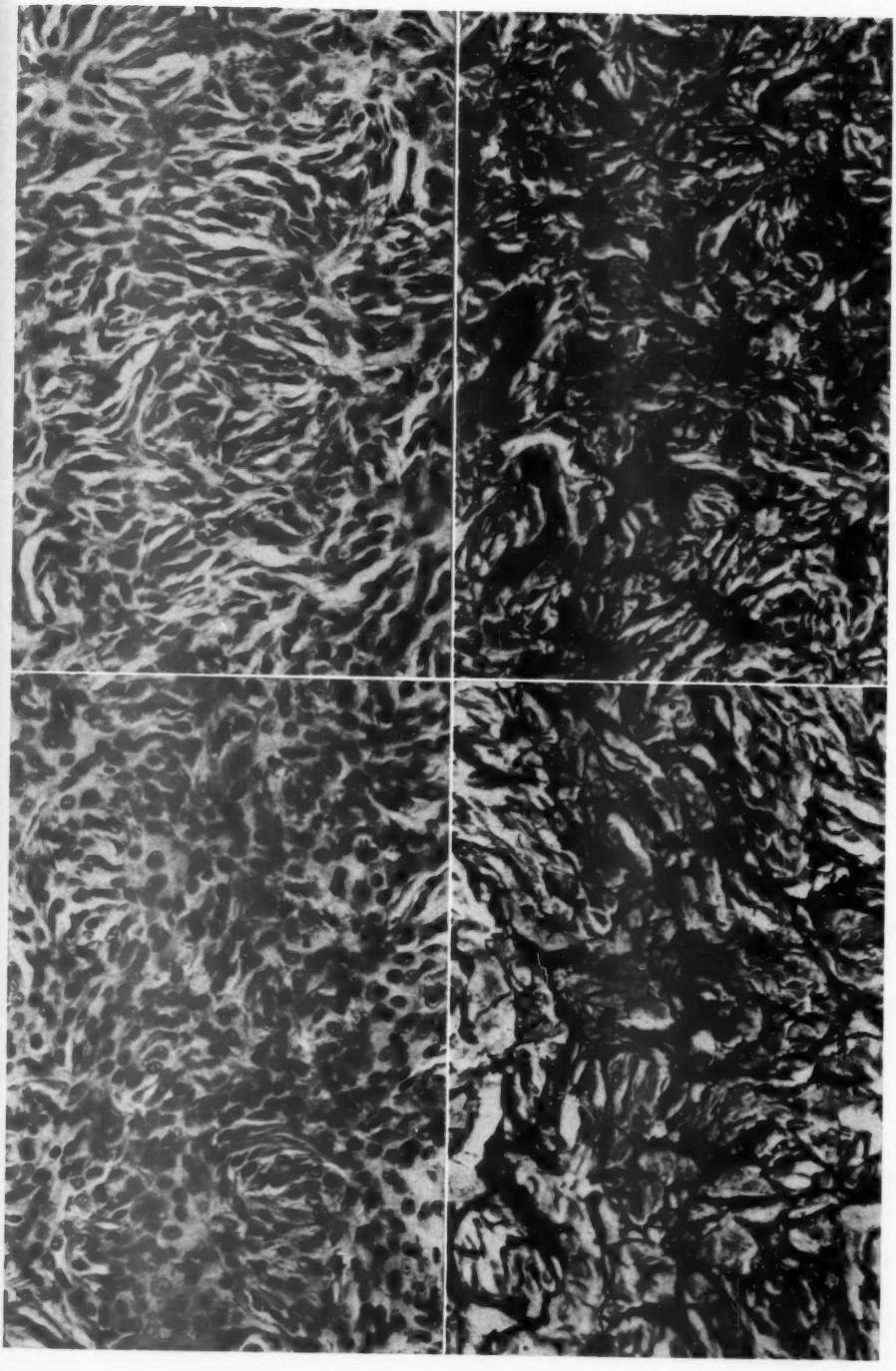
8

FIG. 9. Hemangiopericytoma, 111 cc., 62 gm.; a subcutaneous primary tumor of the left foreleg of a male mongrel shepherd, obtained at necropsy. Hematoxylin and eosin stain. × 330.

FIG. 10. Same tumor as in Figure 9. Wilder's reticulum stain counterstained by aqueous aniline blue. × 330.

FIG. 11. Hemangiopericytoma, 115 cc., 90 gm. This was a subcutaneous tumor on the right shoulder of a male Irish terrier, 9 years old. The primary tumor, which was excised, had been noted for 1 year. Hematoxylin and eosin stain. × 330.

FIG. 12. Same tumor as in Figure 11. Wilder's reticulum stain counterstained with aqueous aniline blue. × 330.



9

10

1

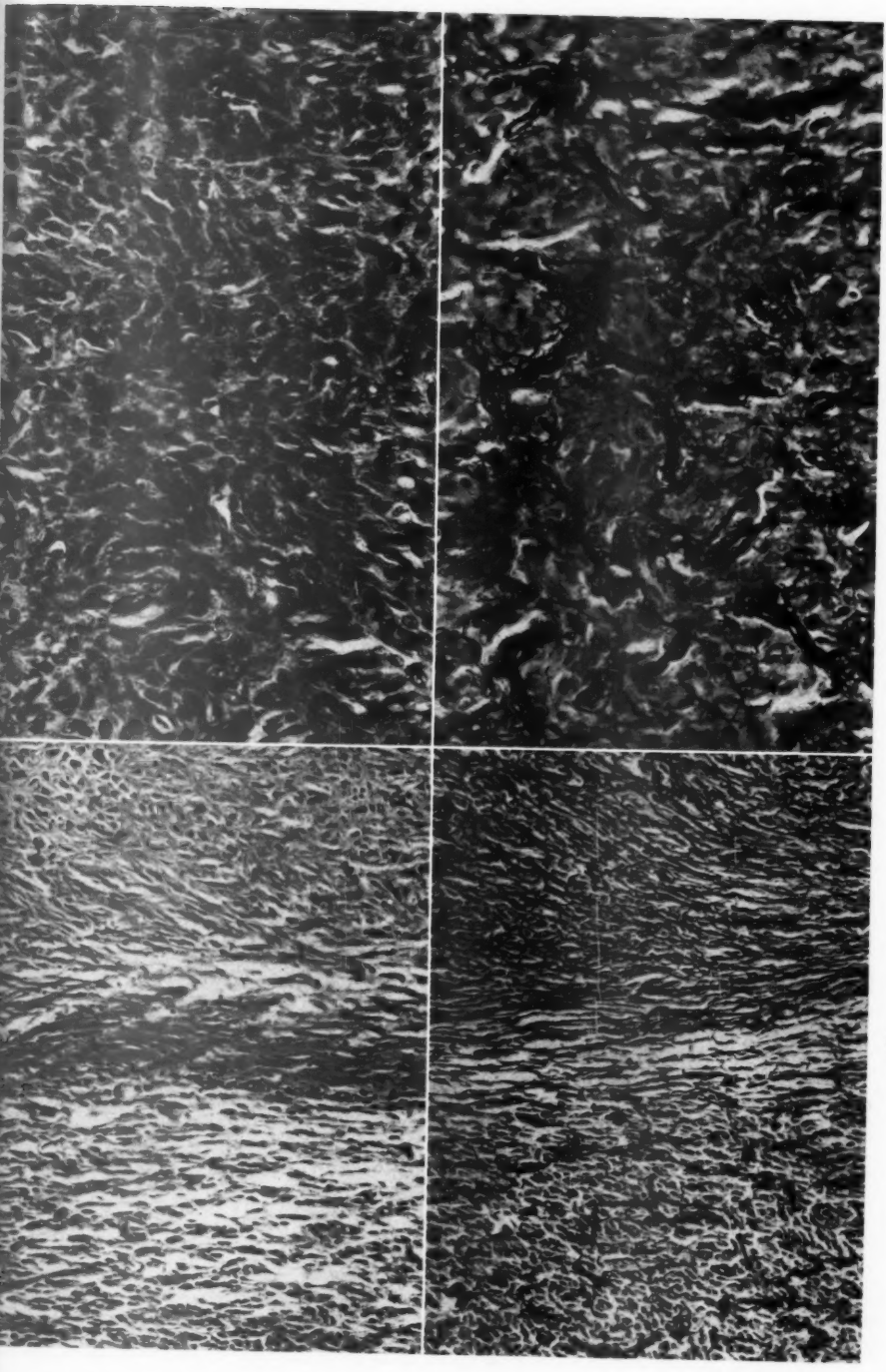
12

FIG. 13. Hemangiopericytoma, 7 cc., 4 gm., of the subcutaneous tissues of the right foreleg, male poodle, 17 years old. This primary tumor had been noted for 4 months. Three recurrences were excised 6, 12, and 20 months later. Hematoxylin and eosin stain. × 330.

FIG. 14. Same tumor as in Figure 13. Wilder's reticulum stain counterstained with aqueous aniline blue. × 330.

FIG. 15. First recurrence, 24 cc., 20 gm., of the tumor in Figure 13, excised 6 months after removal of primary tumor. Loss of capillary pattern in recurrent neoplastic tissue in upper half. Retention of capillary pattern seen in Figure 14 in residual primary tumor in lower half. Hematoxylin and eosin stain. × 160.

FIG. 16. Same tumor and orientation as in Figure 15. Loss of capillary pattern in upper half. Retention of capillary pattern in lower half. Wilder's reticulum stain counterstained with aqueous aniline blue. × 160.



14

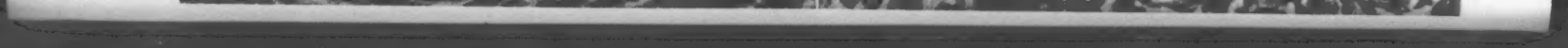
16

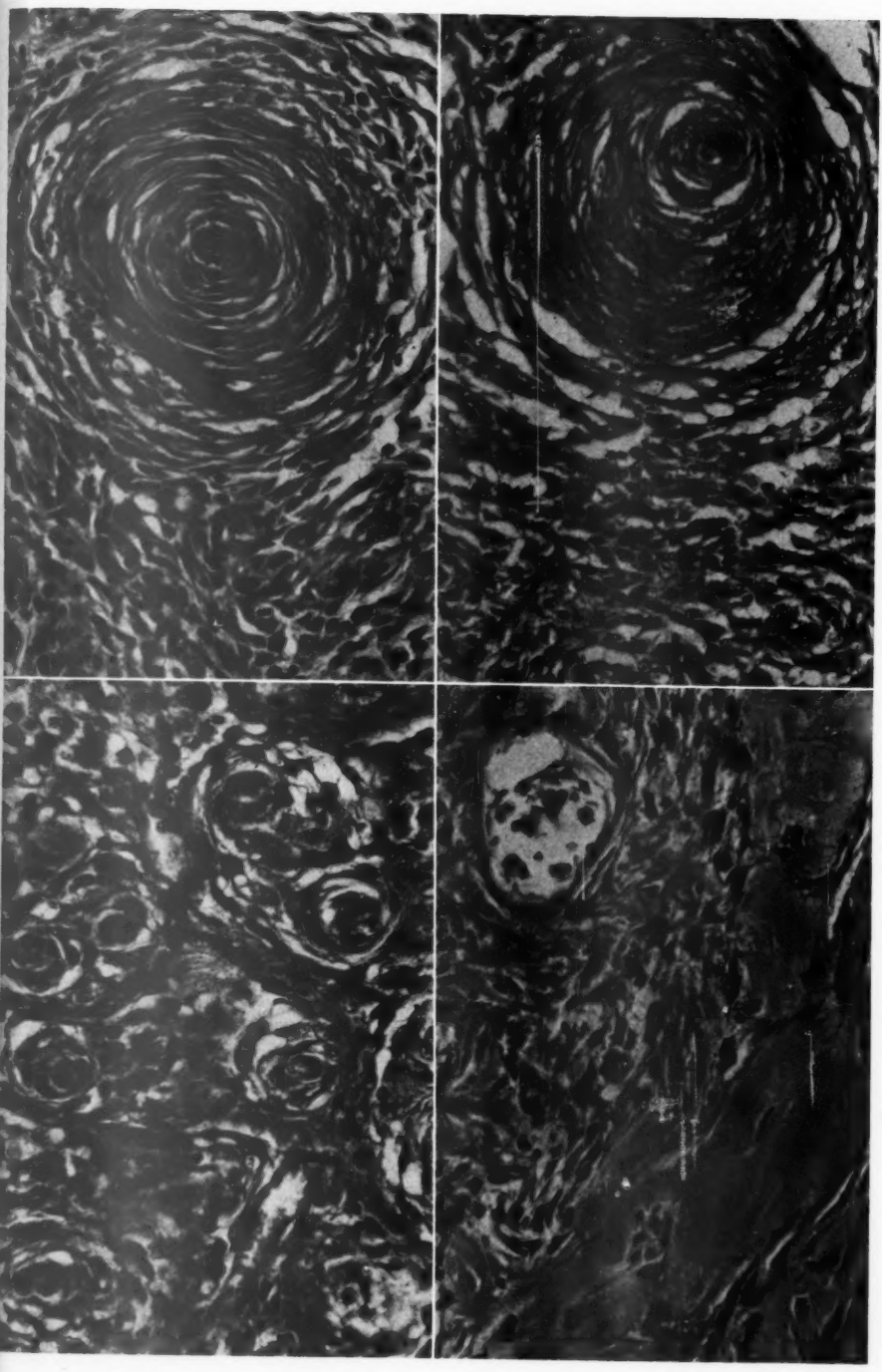
FIG. 17. Hemangiopericytoma, "size of soft ball." This was a subcutaneous tumor of the elbow of a female mongrel German shepherd, 8 years old. This neoplasm recurred to a larger size 1 year later. "Onion skin" pattern of pericytes in large structure in upper half. Hematoxylin and eosin stain. × 330.

FIG. 18. Same tumor as in Figure 17. Wilder's reticulum stain counterstained with aqueous aniline blue. × 330.

FIG. 19. Subcutaneous hemangiopericytoma, 56 cc., of the left gluteal region of a female fox terrier, 10 years old. No recurrence 1 year later. Gomori's trichrome stain. × 330.

FIG. 20. Hemangiopericytoma, 15 cc. This was a jejunal tumor of a spayed female Boston terrier, 11 years old, obtained at necropsy. There had been melena for 2 months before death. Severe sclerosis with collars of collagen fibrils around capillaries. Hematoxylin and eosin stain. × 330.





18

20